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JESUS RODRIGUES DOMINGOS DE SOUZA

**TAXONOMIA INTEGRATIVA DE *ALLOBATES* (ANURA:
AROMOBATIDAE) DO SUDOESTE DA AMAZÔNIA BRASILEIRA, COM
DELIMITAÇÃO DE TRÊS ESPÉCIES NOVAS**

Manaus, Amazonas

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do título de Doutor em Zoologia.

Orientadora: Albertina Pimentel Lima

Coorientador: Igor Luis Kaefer

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Sinopse:

Nesta tese, fez-se uso de diferentes linhas de evidência para a compreensão dos limites entre espécies de diminutos sapos diurnos de serrapilheira do gênero *Allobates*. Foi possível descrever duas novas espécies e demonstrar a existência de uma terceira espécie candidata para a região sudoeste da Amazônia. Desse modo, contribuiu-se para a caracterização da elevada riqueza do gênero e para a compreensão de aspectos relacionados à taxonomia, sistemática e história evolutiva do grupo.

Palavras-chave: Acre, Amazônia, Amphibia, Anura, *Allobates*, bioacústica, biologia molecular, morfologia, taxonomia

*Dedico esta tese aos meus avós, José Domingos e Maria do Rosário (Lia) (**'In memoriam'**), a meu pai, João Rodrigues (**'In memoriam'**) e a minha mãe, Djanira Domingos, por tudo o que eles representam para mim, e em agradecimento, por um dia terem me permitido a vida, sua beleza e o que realmente vale a pena numa existência.*

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“A primeira parte do conhecimento é o nome certo”.

(Provérbio chinês)

RESUMO

Anuros do gênero *Allobates* (Dendrobatoidea, Aromobatidae) compõem um grupo monofilético de pequenos habitantes da serrapilheira de florestas tropicais que se distribuem do oeste do Andes ao leste do Brasil. O principal desafio para a alfa taxonomia deste gênero relaciona-se com o pronunciado conservatismo de caracteres da morfologia externa entre as espécies, os quais são usualmente utilizados como diagnósticos e que contrastam fortemente com uma notável disparidade bioacústica e genética entre populações. Utilizando o que convencionou-se chamar de taxonomia integrativa, método abrangente para delimitar e descrever táxons integrando informação de diferentes classes de caracteres, investigou-se linhagens de *Allobates* do sudoeste da Amazônia, estado brasileiro do Acre, com uso de dados moleculares, bioacústicos e morfológicos de adultos e girinos. **No primeiro capítulo**, descrevemos *Allobates velocicantus* para a região do Juruá, a qual é de extrema importância para a conservação de vários grupos biológicos. Este táxon diferencia-se das demais espécies de *Allobates* genética e morfolologicamente, mas principalmente pelo canto de anúncio, com estrutura inédita para o gênero. **No segundo capítulo**, descrevemos *Allobates albiventris* para a região de cabeceiras do rio Purus. Esta espécie foi confundida e nomeada erroneamente, por um longo período como *Allobates gasconi* em diversos locais na Amazônia. **No terceiro capítulo**, descrevemos uma nova espécie pertencente ao complexo *trilineatus*, amplamente distribuído na Amazônia e que tem sua diversidade subestimada, com várias espécies erroneamente determinadas e que carecem de descrição formal. Esta tese contribui não somente para a taxonomia de *Allobates*, mas também para uma melhor compreensão da evolução e biogeografia da ainda subestimada biodiversidade do sudoeste da Amazônia.

Palavras-chave: Acre, Amphibia, Bioacústica, Biologia molecular, Dendrobatoidea, Inambari

ABSTRACT

Anurans of the genus *Allobates* (Dendrobatoidea, Aromobatidae) comprise a monophyletic group of small leaf litter inhabitants of tropical forests, which are distributed from the western Andes to eastern Brazil. The main challenge for the alpha taxonomy of this genus is related to the pronounced conservatism of external morphological characters between species, which are usually used as diagnoses and which strongly contrast with a remarkable bioacoustic and genetic disparity among populations. Using what is conventionally called integrative taxonomy, a comprehensive method to delimit and describe taxa integrating information from different classes of characters, *Allobates* lineages from southwestern Amazonia, in the Brazilian state of Acre, were investigated using molecular, bioacoustic, and morphological data of adults and tadpoles. **In the first chapter**, we describe *Allobates velocicantus* for the Juruá region, which is extremely important for the conservation of several biological groups. This taxon is genetically and morphologically different from the other species of *Allobates*, notably by its advertisement call, which is considered structurally unprecedented for the genus. **In the second chapter**, we describe *Allobates albiventris* for the headwaters of the Purus river. This species was confused and misnamed, for a long time, as *Allobates gasconi*, in several Amazonian localities. **In the third chapter**, we describe a new species belonging to the *trilineatus* complex, widely distributed in Amazonia and whose diversity is underestimated, with several species erroneously determined and lacking formal description. This thesis contributes not only to the taxonomy of *Allobates*, but also to a better understanding of the evolution and biogeography of the still underestimated southwestern Amazonian biodiversity.

Key-words: Acre, Amphibia, Bioacoustics, Molecular biology, Dendrobatoidea, Inambari

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INTRODUÇÃO GERAL

Taxonomia é a disciplina que identifica, descreve e nomeia espécies e outros taxa atuais e extintos, além de estar intimamente ligada às suas classificações em contextos mais amplos (Simpson, 1961; Mayr, 1982; Padial et al., 2010; Pires & Marinoni, 2010). Ela representa o braço mais ancestral da biologia, tão antiga quanto a linguagem humana e desenvolveu-se de forma independente em diferentes regiões do planeta. Não é difícil imaginar que civilizações humanas antigas ao identificar, nomear e classificar espécies temidas, comestíveis ou venenosas, produziam conhecimento taxonômico, os quais eram transmitidos de forma oral às gerações seguintes (Harari, 2015). Durante muito tempo, ao longo da história humana, os registros taxonômicos ficaram restritos às regiões de origem, sendo acessados somente tempos depois, com as grandes navegações, o comércio entre as nações e o avanço nos meios de comunicação. Notadamente, o conhecimento taxonômico ocidental desenvolveu-se alheio às descobertas taxonômicas do oriente, que alcançou as demais regiões somente a partir da idade média (Manktelow, 2008).

Os registros taxonômicos mais antigos são de origem chinesa. A farmacopeia “*Divine Husbandman's Materia Medica*” escrita por Shen Nung, imperador da China (3.000 a. C.), considerado o pai da medicina chinesa, ensina 365 medicinas derivadas de minerais, plantas e animais. Além dos chineses, o conhecimento egípcio (1.500 a. C.), por sua vez, representado por ilustrações em muros e “*papyrus*”, usos de vários tipos de plantas na medicina, bem como os processos de mumificação, configuram-se como importantes contribuições antigas ao conhecimento taxonômico (Morton, 1981; Manktelow, 2008).

Contribuições fundamentais à taxonomia vieram da Grécia e Roma antigas. Aristóteles (384–322 a. C.), o mais célebres entre os gregos, foi o primeiro a classificar as coisas vivas; Theophrastus (370–285 a. C.), estudante de Platão e Aristóteles publicou “*Historia Plantarum*” com 480 espécies de plantas e Dioscorides (40–90 a. C.), um físico, que tinha interesse em plantas medicinais, publicou “*Materia Medica*”, com cerca de 600 espécies. Entre os romanos destaca-se Plinius (23–79 a. C.) com a obra “*Naturalis Historia*”, que contém 160 volumes nos quais descreve várias plantas em latim. Plinius é considerado o pai do latim botânico (Mayr, 1982; Manktelow, 2008).

Ao final do século XVI, os trabalhos taxonômicos originais começaram a substituir obras mais antigas. Uma das razões foi o desenvolvimento de lentes ópticas, que possibilitou a

observação em detalhes de diferentes aspectos morfológicos, em diferentes espécies. Nesta época destacam-se os autores Caesalpino (1519–1603) na Itália, que em sua obra “*De Plantis*”, classificou espécies quanto ao hábito de crescimento e a forma do fruto e semente; os irmãos suíços Bauhin (1541–1631; 1560–1624), autores do “*Pinax Theatri Botanici*”; o naturalista inglês John Ray (1627–1705), que publicou muitas obras e deu importantes contribuições, dentre elas o estabelecimento de espécie como a última unidade taxonômica; e o francês Joseph Pitton de Tournefort (1656–1708), que baseou sua classificação em caracteres florais, o qual foi utilizado por Linnaeus, posteriormente (Morton, 1981; Manktelow, 2008).

A partir do século XVI, com a revolução científica em curso, novidades no campo da taxonomia aconteceram. As mais impactantes foram as contribuições de Carolus Linnaeus (1707–1778) que percebeu a importância de uma taxonomia universal, fundamentada nas características e relações vitais entre os seres. Sua inovação técnica, a qual incluiu a introdução de um sistema rigoroso de diagnose, padronização de sinônimas e demais inovações conceituais na pesquisa, trouxe simplicidade e uniformidade à taxonomia. Em seu trabalho *Species Plantarum* (1753), descreveu 7.300 espécies de plantas com seu sistema binomial revolucionário, que utilizava gênero e espécie em latim, para cada organismo, refletindo sempre que possível as características da planta. Posteriormente, em *Systema Naturae* (1758), criou uma organização divisional e hierárquica de classificação, com Classe, Ordem, Gênero e Espécie. Seu sistema de classificação foi amplamente aceito, dando origem a taxonomia moderna e é a base da classificação hierárquica atual.

A taxonomia pós-lineana teve grande influência de cientistas franceses que propuseram novos métodos naturais de classificação. Entre os franceses destacam-se Georges-Luise Leclerc de Buffon (1707–1788), que preferia descrever o mundo ao invés de classificá-lo; Michel Adanson (1727–1806), que defendia o uso de mais caracteres, ao invés de ênfase em alguns; Antoine Laurent de Jussieu (1748–1836), que classificou as plantas quanto a sua morfologia em acotiledôneas, monocotiledôneas e dicotiledônias e; Jean-Baptiste de Lamarck (1744–1829), que lançou uma teoria evolutiva baseada na herança dos caracteres herdados (Morton, 1981; Mayr, 1982; Manktelow, 2008).

As contribuições de Darwin (1859), bem como as de Alfred Russel Wallace (1823–1913), porém, foram as mais decisivas para a teoria da classificação da Biota. Esses autores reportaram a existência de grupos naturais e do provável mecanismo pelo qual compartilham caracteres. Além disso, Darwin demonstrou de forma clara que os membros de um táxon geralmente são semelhantes por descenderem de um ancestral comum. Para Mayr (1982), a

teoria de descendência comum de Darwin forneceu uma explicação teórica para a hierarquia de Linnaeus, bem como para a homogeneidade do táxon em uma classificação natural, restaurando o conceito de continuidade entre as espécies. Contudo, pouca coisa mudou em sistemática, até as contribuições de Ernst Haeckel (1834–1919) e August Wilhelm Eichler (1839–18878), que iniciaram a construção de árvores evolutivas, dando origem ao termo “filogenia”. Nesta época, em regra, observa-se as similaridades e diferenças para criar sistemática (Manktelow, 2008). Com o surgimento da cladística, com Willi Hennig (1913–1976), uma nova era iniciava, onde apenas as similaridades agrupam espécies e o taxa poderia incluir todos os descendentes de um único ancestral (monofilia), possibilitando assim, teste de hipóteses em sistemática (Dupuis, 1984). Esse método ainda passaria por muita controvérsia até a popularização do DNA e o uso de programas de computadores que possibilitaram manusear grandes bancos de dados, tornando a cladística quase uma regra em sistemática.

De forma teórica geral, em oposição à operacional relacionada a critérios de delimitação, espécies são linhagens de populações e metapopulações que evoluem independentemente (Queiroz, 2005; 2007) e representam a unidade central para a taxonomia (Padial et al. 2010). Não resta dúvida que vincular espécie a um nome é a única forma de assegurar que estamos falando da mesma entidade, podendo assim associar todas as informações obtidas, das mais variadas formas, em análises comparativas, com confiabilidade (Schlick-Steiner et al., 2007; Patterson et al., 2010; Padial et al., 2010; Satler et al., 2013). Dada a extrema particularidade das espécies e quão pouco sabemos sobre elas—sobretudo as que podem ser as mais importantes no funcionamento dos ecossistemas (La Salle et al., 2009), a taxonomia desempenha um papel central para o conhecimento de padrões e processos referentes ao passado, presente e futuro da biodiversidade, fornecendo a base de dados necessária para todas as demais ciências biológicas (Schlick-Steiner et al., 2010; Wilson, 2004).

Desde a revolução conceitual iniciada por Linnaeus, catalogou-se cerca de 23% da biodiversidade do planeta, da qual cerca de 14% espécies são terrícolas e 9% espécies são marinhas, em uma estimativa total de 8.7 milhões de espécies (Mora et al., 2011). Considerando-se esse ritmo, serão necessários cerca de 303 mil taxonomistas, por não menos que 1.200 anos de trabalho, com um custo aproximado de 364 bilhões de dólares para que se conheça toda a riqueza biológica do planeta (Mora et al. (2011). Padial et al. (2010) estimou um período de tempo de cerca de 400 anos, dados os recursos atualmente empregados, enquanto Wheeler et al. (2004) e Costello et al. (2013) estimaram um custo financeiro de 0.5–1.0 bilhão de dólares por ano para aumentar de forma efetiva o esforço taxonômico global ao longo dos

próximos 50 anos. Dadas as estimativas de taxas de extinção, de 20–200 espécies/milhão de espécies/ano, as quais correspondem de 100–1.000 vezes as taxas de extinção pré-humanos (Pimm et al., 1995), milhares de espécies serão extintas antes mesmo que possamos nomeá-las (Pimm et al., 2006).

Apesar de sua importância, a taxonomia passa por uma grave crise (Wheeler et al., 2004; Wilson, 1985, 2003) caracterizada principalmente pelo crescimento das demais disciplinas biológicas e pelo desinteresse por taxonomia. Enquanto outras disciplinas observaram incrementos em investimento, a taxonomia amargou a redução de seus fundos de pesquisa, ausência de especialistas em vários grupos e áreas geográficas e a desvalorização e desprestígio dos seus profissionais (Godfray, 2002; Mallet & Willmott, 2003; Pires & Marinoni, 2010). Adicionalmente, muitas vezes a taxonomia é tachada como uma ciência obsoleta, pouco atrativa, meramente descritiva, limitada a periódicos científicos de baixo impacto (Carbayo & Marques, 2011). Este panorama contribui para o chamado “impedimento taxonômico”, o qual pode ser definido como a ausência do conhecimento taxonômico ou a impossibilidade de cumprir com a tarefa de descrever a biodiversidade, trazendo consequências negativas para diversas subdisciplinas como biologia evolutiva, biogeografia e biologia da conservação (Wheeler et al., 2004).

Como um meio de revitalizar a tradicional taxonomia e ajudá-la a superar a crise taxonômica, algumas abordagens alternativas e complementares foram propostas, dentre as quais figura o uso de *DNA barcoding* (Hebert et al., 2003), o qual tem sido bem-sucedido para operacionalizar a identificação e delimitação de novas espécies em vários grupos biológicos (e.g., Hebert et al., 2004; Ward et al., 2005; Cywinska et al., 2006). Este método, o qual pode ser considerado como uma ferramenta taxonômica operacional, recebeu muitas críticas por grande parte da comunidade científica, alegando-se desvio de fundos para outras disciplinas e por representar uma ameaça à taxonomia tradicional, até então baseada majoritariamente em morfologia (e.g., Cameron et al., 2006; Savolainen et al., 2005). Entretanto, o debate proposto por Hebert et al. (2003), expôs a necessidade de modernização da taxonomia. As limitações da taxonomia tradicional baseada somente em morfologia pontuadas pelo autores foram: (a) plasticidade fenotípica dos caracteres empregados para reconhecimento das espécies levando a incorretas identificações; (b) espécies crípticas morfologicamente sendo frequentemente subestimadas; (c) inexistência de chaves e guias para identificar imaturos de muitas espécies e (d) requerimento de alto nível de “*expertise*” pela taxonomia tradicional, tornando-a restrita a

especialistas. Em adição, cada vez menos especialistas estão disponíveis para a árdua e gigantesca tarefa de descrever a biodiversidade global.

Por outro lado, a taxonomia molecular empregada de forma isolada, mostrou-se insuficiente para superarmos a crise taxonômica. Tautz et al. (2003) indicam de maneira bastante clara que o uso de abordagens moleculares, integradas com o sistema taxonômico tradicional e demais possibilidades tecnológicas constituem a forma mais rápida e segura de obtermos sucesso em revelar a biodiversidade da Terra. Em suas palavras *“chegou o momento em que o conhecimento molecular e morfológico pode e deve ser formal e frutuosamente combinado”*.

Recentemente, temos testemunhado um crescimento em grande escala, do que se convencionou chamar taxonomia integrativa. De acordo com Pante et al. (2015), essa abordagem foi introduzida formalmente e de forma independente por Dayrat (2005) e Will et al. (2005). Trata-se de um método abrangente para delimitar e descrever táxons integrando informações provenientes de diferentes classes de caracteres, e consequentemente de procedimentos técnicos, fazendo uso conjunto de diferentes critérios operacionais para delimitação de espécies sem confundi-lo com o longo debate acerca de conceitos de espécie. Desse modo, percebemos que o uso de dados moleculares revelou-se uma ferramenta útil tanto para desvendar a diversidade críptica—duas ou mais espécies morfológicamente similares, erroneamente classificadas (e escondidas) sob um mesmo nome, em vários grupos taxonômicos (Funk et al., 2012)—quanto delimitação e hipótese de espécies, fazendo conexão com métodos clássicos e permitindo que a taxonomia desenvolvesse uma interface sinérgica com as mais variadas áreas do conhecimento (Miller, 2007).

Mesmo com o debate caloroso sobre a hierarquia dos caracteres e os critérios a utilizar para delimitar espécies (Padial et al., 2010; Schlick-Steiner et al., 2010; Yeates et al., 2011), a maioria dos taxonomistas concorda que avaliar várias linhas de evidência é a abordagem mais eficiente e teoricamente fundamentada para a proposição de hipóteses de delimitação de espécies. Desde então, a aplicação de métodos moleculares à taxonomia tradicional tem revolucionado a descoberta e descrição da biodiversidade.

Neste contexto, DeSalle et al. (2005) oferecem a visão do ciclo taxonômico como uma representação da taxonomia contemporânea. Tal visão foi corroborada por Padial et al. (2010) (Fig. 1), os quais reforçam a importância da adoção de procedimentos transdisciplinares e novas ferramentas voltadas para a inovação tecnológica e novas formas de comunicação, divulgação e disponibilização do conhecimento taxonômico. Os princípios da taxonomia integrativa são:

(i) qualquer tipo de dado é potencialmente relevante para documentar as diferenças entre espécies, (ii) diferentes tipos de dados são complementares e destacam diferentes aspectos do processo de especiação, (iii) taxonomistas podem obter quantas linhas de evidências independentes quanto possível, (iv) dados taxonômicos podem ser integrados, e (v) avaliações taxonômicas podem ser revisitadas quando novos dados tornarem-se disponíveis (Padial et al., 2010; Yeates et al., 2011; Sangster, 2018).

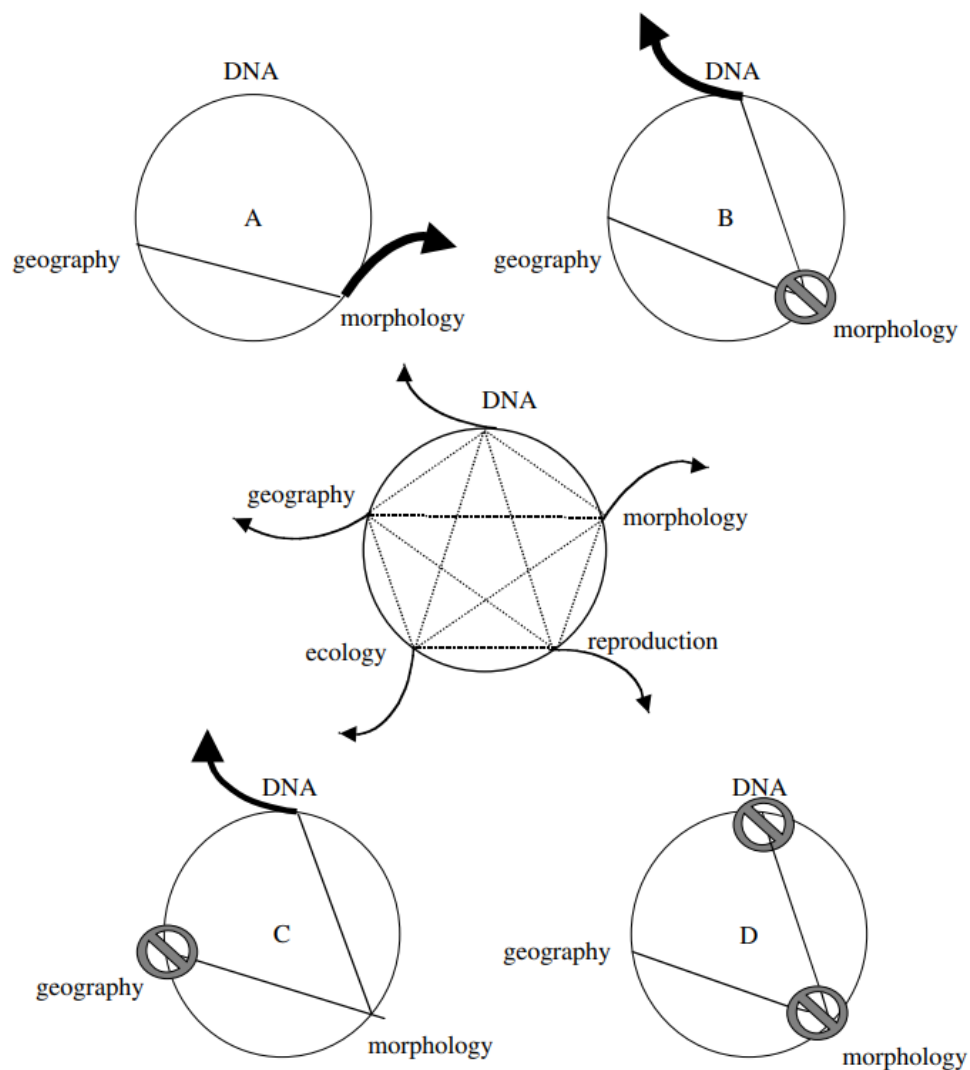


Figura 1. Ciclo taxonômico. (A) Taxonomia clássica. Hipótese taxonômica estabelecida com base em organismos que parecem similares em uma localidade geográfica. A hipótese taxonômica é testada com informação morfológica e corroborada com atributos morfológicos. (B) Espécies crípticas. Uma hipótese geográfica é colocada e testada através de caracteres morfológicos. Os atributos morfológicos não corroboram a hipótese geográfica e o taxonomista não pode sair do ciclo. Mantendo a hipótese geográfica, o taxonomista então examina os agregados usando a hipótese geográfica com sequências de DNA. A corroboração então segue com os caracteres da sequência de DNA sendo diagnósticos. (C) Espécies simpátricas.

Diferenças morfológicas são reconhecidas entre um grupo de organismos. A hipótese de agregação é proposta com base na informação morfológica. A distribuição geográfica é usada para testar padrões de agregação. Quando a distribuição geográfica é usada para testar o padrão de agregação, não existe padrão geográfico para a distribuição de diferentes tipos morfológicos. O taxonomista então usa atributos da sequência de DNA para testar a hipótese morfológica de agregação e corrobora a hipótese morfológica. O taxonomista sai do círculo. **(D)** Falha em detectar um novo táxon; neste exemplo, uma hipótese geográfica é testada com informação morfológica. A informação morfológica falha e a hipótese geográfica e qualquer outra hipótese morfológica de agregação são testadas com informação de sequências de DNA. Sequência de DNA falha em rejeitar a hipótese de não haver nova taxa, o taxonomista não pode sair do ciclo e a inferência é que não há diferença nos agregados, portando apenas um único táxon. Adaptado de Padial et al. (2010).

Nos últimos anos um expressivo número de estudos envolvendo abordagens integrativas em diversos grupos taxonômicos tem sido publicado. Este método de trabalho tem contribuído para uma melhor compreensão de diversos aspectos como história biogeográfica, diversidade críptica e descrição de novas espécies de diversos táxons como aves (e.g., Ribas et al., 2012; Wei et al., 2022), peixes (e.g., Garavello et al., 2021; Pugedo et al., 2016), répteis (e.g., Streicher & Meik, 2018) e anfíbios (e.g., Batista et al., 2016; Ferrão et al., 2016; Toledo & Batista, 2012).

A taxonomia integrativa tem sido extremamente importante em grupos que possuem maior vulnerabilidade às ameaças de extinção. Um exemplo é Amphibia, o grupo de vertebrados mais ameaçado do planeta (IUCN, 2022). Estes organismos normalmente apresentam pequeno tamanho corporal, baixa capacidade de dispersão e elevada dependência de condições ideais de temperatura, umidade e disponibilidade de água. O aumento da pressão causada pela modificação de habitats e mudanças climáticas provavelmente constituem os maiores riscos aos anfíbios, os quais são potencializados em espécies estreitamente adaptadas (i.e. especialistas) e com âmbito geográfico restrito (Wake & Vredenburg, 2008). O declínio populacional em diversas regiões do planeta (Blaustein & Wake, 1990; Stuart et al., 2004; Pyron & Wiens, 2011; Catenazzi, 2015) e a estimativa de que cerca de 40% das espécies de anfíbios estão sob algum risco de ameaça, indicam a alta vulnerabilidade deste grupo, o qual pode estar experienciando um evento de extinção em massa (IUCN, 2022).

Anuros do gênero *Allobates* Zimmermann & Zimmermann, 1988, compõem um grupo monofilético de pequenos habitantes da serrapilheira de florestas tropicais, que se distribuem do oeste do Andes, na América Central à Mata Atlântica brasileira na América do Sul, incluindo a região do Caribe e excetuando-se Argentina, Chile, Paraguai e Uruguai (Grant et al., 2006, 2017; Frost, 2023). O crescente interesse nestes anuros tem gerado notável conhecimento acerca

das espécies, especialmente após as contribuições de Morales (2002), Grant et al. (2006) e Santos et al. (2009). Desde então a taxonomia e sistemática do grupo passou por grandes avanços, com descrição de diversas novas espécies (e.g., Lima et al., 2007; Kok et al., 2013; Simões, 2016; Simões et al., 2010, 2019; Melo-sampaio et al., 2020), redescrições de espécies cuja caracterização modesta dificultava a diferenciação entre exemplares (Caldwell et al., 2002; Lima et al., 2009), novas filogenias melhorando a resolução de espécies crípticas, com subsequente descrições ou redescrições (Simões et al., 2010; Melo-Sampaio et al., 2018; Lima et al., 2020; Jaramillo et al., 2021; Gagliardi-urruetia et al., 2021; Ferrão et al., 2022) e importantes atualizações em sistemática filogenética e biogeografia histórica (Grant et al., 2006; Grant et al., 2017; Réjaud et al., 2020).

O principal desafio para a alfa taxonomia em *Allobates* deve-se ao pronunciado conservatismo da morfologia externa (Kaefer et al., 2013; Fernandes et al., 2021) e o padrão de cores entre as espécies, caracteres usualmente utilizados como diagnósticos (Grant et al., 2006; 2017). De forma geral, essa tendência conservadora na morfologia externa dos anfíbios contribui para uma alta relevância de sinais não visuais, como é o caso dos sinais acústicos (Bickford et al., 2007). O fato de que mudanças morfológicas não necessariamente correspondem às divergências genéticas profundas que caracterizam processos de diversificação (Kaefer et al., 2013; Maia et al., 2017), ressalta a importância do acesso à notável diversidade bioacústica e genética do gênero. Contudo, a integração de variadas fontes de dados, tais como bioacústicos, morfológicos, e especialmente DNA mitocondrial (Padial et al., 2010), têm ajudado a testar hipóteses de novas espécies (e.g., Moraes et al., 2019; Lima et al., 2020; Jaramillo et al., 2021; Ferrão et al., 2022) e melhorar a resolução sistemática dentro do gênero (Grant et al., 2006; 2017; Santos et al., 2009; Réjaud et al., 2020).

A disponibilidade de amostras, bem como crescente utilização de dados moleculares, tem possibilitado ainda o teste de hipóteses biogeográficas (Gascon et al., 1998; Loughheed et al., 1999; Maia et al., 2017), proposição de modelos de diversificação para o grupo (Santos et al., 2009; Réjaud et al., 2020), estudos de genética de paisagem (Ferreira et al., 2020) e de variação genética e fenotípica em relação ao gradiente ambiental e latitudinal (Fernandes et al., 2021). Além disso, estudos comportamentais (e.g., Da Rocha et al., 2017; Ursprung et al., 2011), bioacústicos (Bastos et al., 2011; Chaloupka et al., 2022; Forti et al., 2017; Rodríguez & Hödl, 2020; Simões et al., 2008) e ecológicos (Dias-Terceiro et al., 2015; Ferreira et al., 2018) têm contribuído ao conhecimento sobre padrões e processos responsáveis pela excepcional riqueza deste gênero, especialmente no bioma amazônico.

Allobates atualmente é composto por 60 espécies nominais, sendo 65% destas, descritas muito recentemente, ainda no século XXI (Frost, 2023). A maior riqueza de espécies está distribuída nas terras baixas amazônicas e regiões de transição para altas montanhas andinas, ao sul do rio Solimões-Amazonas (Melo-Sampaio et al., 2018; Frost, 2023). Aproximadamente 47% das espécies descritas ocorrem nesta região, com destaque para a região sudoeste da Amazônia, nos interflúvios Purus-Madeira e cabeceiras dos rios Purus, Juruá e Ucayali, com alta diversidade alfa, correspondendo à cerca de 27% das espécies conhecidas (Frost, 2023). Pesquisas nesta região, especialmente em áreas pouco exploradas, são de extrema importância para a produção de dados que auxiliem no conhecimento acerca da sistemática, taxonomia e biogeografia do grupo. Além disso, podem ajudar planejamentos de conservação, tais como manutenção e criação de áreas protegidas.

Nesse contexto, a presente tese tem como objetivo geral delimitar linhagens de *Allobates* do sudoeste da Amazônia, por meio de taxonomia integrativa, utilizando dados moleculares, bioacústicos e morfológicos de adultos e girinos e dessa contribuir para compreensão do passado biogeográfico de grupos taxonômicos, em especial o grupo do *Allobates*, bem como a formação, origem e evolução da paisagem amazônica e cenários evolutivos e biogeográficos futuros. Os objetivos específicos do estudo, os quais ditam a organização dos capítulos e manuscritos provenientes da tese são os seguintes:

- (1) Capítulo 1. Delimitação e descrição de uma nova espécie de *Allobates* para a região do Alto Juruá;
- (2) Capítulo 2. Delimitação e descrição de uma nova espécie de *Allobates*, anteriormente confundida e erroneamente classificada como *Allobates gasconi*, e;
- (3) Capítulo 3. Delimitação e descrição de uma nova espécie de *Allobates* pertencente ao complexo *Allobates trilineatus*.

Allobates velocicantus sp. nov.



CAPÍTULO 1

Jesus R.D. Souza, Miquéias Ferrão, James Hanken e Albertina P. Lima. A new nurse frog (Anura: *Allobates*) from Brazilian Amazonia with a remarkably fast multi-noted advertisement call. Artigo publicado no periódico PeerJ em 04 de novembro de 2020. DOI 10.7717/peerj.9979.

A new nurse frog (Anura: *Allobates*) from Brazilian Amazonia with a remarkably fast multi-noted advertisement call

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ABSTRACT

Nurse frogs (Aromobatidae: *Allobates*) are probably the most extensively studied genus by taxonomists in Brazilian Amazonia. The southwestern portion of Amazonia is the most species-rich: as many as seven species may occur in sympatry at a single locality. In this study, we describe a new species of nurse frog from this region. The description integrates data from larval and adult morphology, advertisement calls and DNA sequences. *Allobates velocicantus* sp. nov. is distinguished from other *Allobates* mainly by the absence of hourglass-shaped dark marks on the dorsum and dark transverse bars on the thigh; a throat that is white centrally and yellow marginally; basal webbing on toes II and III; finger I longer than finger II; and an advertisement call composed of 66–138 pulsed notes with a note duration of 5–13 ms, inter-note intervals of 10–18 ms and a dominant frequency of 5,512–6,158 Hz. Tadpoles of the new species have 3–4 short, rounded papillae on the anterior labium, 16–23 papillae on the posterior labium, and a labial keratodont row formula 2(2)/ 3(1). This is the fifth species of *Allobates* described from the state of Acre, southwestern Brazilian Amazonia.

Subjects Biodiversity, Conservation Biology, Taxonomy, Zoology

Keywords Advertisement call, Integrative taxonomy, Morphology, Phylogeny, Pulsed notes, State of Acre, Tadpole

INTRODUCTION

Nurse frogs of the genus *Allobates* Zimmermann & Zimmermann, 1988 inhabit the leaf-litter of Neotropical forests and are small and diurnal. They are distributed from northern Central America to the Atlantic Forest of Brazil (Grant et al., 2006, 2017; Frost, 2020). Currently, the genus comprises 55 nominal species (Frost, 2020), 22 of which are distributed in Brazilian Amazonia. Due to the general lack of substantial morphological differences among species, the integration of multiple independent lines of evidence (e.g., larval and adult morphology, vocalization, breeding behavior, and molecular data) have proven indispensable for the reliable discovery, diagnosis and formal description of new species (Lima et al., 2010; Lima, Simões & Kaefer, 2014, 2015; Simões, Lima & Farias, 2010; Simões et al., 2013; Simões, Rojas & Lima, 2019).

Because of its high degree of both species richness and hidden diversity, *Allobates* has attracted intense interest by systematists; *Allobates* may represent the most extensively studied genus of anurans in Brazilian Amazonia for the last two decades, a period in which 36 of the 55 species in the genus were described (Frost, 2020). Two to four species frequently cooccur in sympatry in several portions of Brazilian Amazonia, for example, Porto Walter (Grant et al., 2006; Melo-Sampaio, Oliveira & Prates, 2018) and Careiro (Lima et al., 2010; Lima, Ferrão & Silva, in press), in the states of Acre and Amazonas, respectively. However, southwestern Brazilian Amazonia harbors the highest level of species richness, with up to seven species sympatric at some localities, for example, the upper Madeira River (Dias-Terceiro et al., 2015). Six nominal species are known from the state of Acre (*A. femoralis* sensu lato, *A. flaviventris*, *A. gasconi*, *A. hodli*, *A. subfolionidificans* and *A. trilineatus* sensu lato: Lima, Sanchez & Souza, 2007; Simões, Lima & Farias, 2010; Melo-Sampaio, Souza & Peloso, 2013; Melo-Sampaio, Oliveira & Prates, 2018) and several other candidate species await formal description (Grant et al., 2006; Melo-Sampaio, Souza & Peloso, 2013; Grant et al., 2017; Melo-Sampaio, Oliveira & Prates, 2018).

During a herpetological survey of forests along federal highway BR364 and vicinal roads in the state of Acre, A. P. Lima found a species of *Allobates* with a long-lasting call consisting of rapidly emitted notes. We compared this call, as well as the morphology and mtDNA of these specimens, to other described species and concluded that they belong to a yet unnamed species. In the present study, we describe this new species through integrative taxonomy. In addition, we describe its breeding behavior, from courtship to tadpole emergence and transport.

MATERIALS AND METHODS

Sampling. Adult specimens were collected 13–14 January 2019 along state highway AC405 (7°38'54" S, 72°48'59" W, 211 m a.s.l.), municipality of Mâncio Lima, state of Acre, Brazil. Also collected from this locality were a group of tadpoles obtained from the back of INPAH 41347 (field number APL 21403), an adult male, and a clutch of fertilized eggs obtained soon after oviposition. In addition, an adult male was collected 15 February 2019 along the road (7°34'02" S, 72°39'32" W, 181 m a.s.l.) connecting the municipality of Cruzeiro do Sul (state of Acre) to Guajará (state of Amazonas). Adults were euthanized with a 2% benzocaine topical solution, fixed in 10% neutral-buffered formalin and preserved in 70% ethanol. Tissue samples were obtained before immersion in formalin. Tadpoles and eggs were reared in the laboratory until they reached Gosner stages 27–37 for morphological description, when they were euthanized with a 10% aqueous benzocaine solution and preserved in 5% neutral-buffered formalin. Adults and tadpoles were housed in the herpetological section of the Zoological Collection of the Instituto Nacional de Pesquisas da Amazônia (INPAH), Manaus, Amazonas, Brazil. Protocols of collection and animal care follow the Conselho Federal de Biologia resolution number 148/2012 (Conselho Federal de Biologia–CFB, 2012). Specimens were collected under collection permit number 1337-1 provided by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). The advertisement calls of eight males were recorded. Seven males were recorded in the state of Acre: six along highway AC405 (7°38'54" S, 72°48'59" W, 211 m a.s.l.) and one along the road connecting the municipality of Cruzeiro do Sul–Guajará (7°34'02" S, 72°39'32" W, 181 m a.s.l.). The eighth male was recorded near the municipality of Guajará (7°27'10" S, 72°35'14" W, 225 m a.s.l.), Amazonas, Brazil. Recordings were made between 06:30 h and 11:00 h and between 15:00 h and 17:30 h. Air temperature at the time of recording, measured with a digital thermohygrometer positioned 1 m above the ground, ranged from 24 to 27.4 °C. Calls were recorded with a directional microphone Shotgun CSR Yoga HT-81 accoupled to a Zoom H4n digital recorder. The microphone was positioned approximately 1 m in front of the focal active male. Recordings were stored with 16 bits resolution, 44.1 kHz and WAV format. Call recordings were deposited in the Fonoteca Neotropical Jacques Viellard (FNJV 45469–79), UNICAMP, Campinas, Brazil.

Sequencing and phylogenetic analysis. Total genomic DNA was extracted from tissue samples of five specimens of the new species using the commercial kit Wizard (Promega Corp., Madison, WI, USA) following the manufacturer's instructions. Fragments of the 16S rRNA mitochondrial gene were amplified by polymerase chain reaction (PCR) using universal primers

16sar (5'-CGCCTGTTTATCAAAAACAT-3') and 16sbr (5'-CCGGTCTGAACTCAGATCACGT-3')(Palumbi, 1996). Extractions, PCR reactions and sequencing were conducted with both forward and reverse primers (Maia, Lima & Kaefer, 2017). Sequences were visually checked and manually edited in Geneious 5.3.4 (Kearse et al., 2012). Sequences were deposited at GenBank and can be accessed under numbers MT446458–62. We used BLAST to search for sequences already deposited in GenBank that might correspond to the new species. Two 16S rRNA sequences, KY886579 and MF624181, matched our sequences with high identity. Additionally, we retrieved 68 additional 16S sequences that represent the 37 species of *Allobates* currently available in GenBank (Table S1). Sequences of four species of other genera in the family Aromobatidae were used to root the tree: *Anomaloglossus stepheni* (Martins, 1989), *Aromobates nocturnus* Myers, Paolillo & Daly, 1991, *Mannophryne collaris* (Boulenger, 1912) and *Rheobates palmatus* (Werner, 1899). For phylogenetic reconstruction, we downloaded additional sequences of three other mitochondrial genes (12S ribosomal RNA; cytochrome oxidase I, COI; and cytochrome b, cyt b) and six nuclear genes (28S ribosomal RNA, 28S; histone H3, HH3; recombination activating gene 1, RAG1; rhodopsin, RHO; seventh in absentia, SIA; and tyrosinase, TYR) from the same specimens (when available) for which 16S sequences were initially downloaded. Sequences were aligned in Bioedit 7.2.5 (Hall, 1999) using the CLUSTAL W algorithm (Thompson, Higgins & Gibson, 1994). Alignments were concatenated in Mesquite 3.04 (Maddison & Maddison, 2015), which yielded a final alignment consisting of 5,915 base pairs (bp) and 79 terminals. Vouchers and GenBank accession numbers are listed in Table S1.

Table 1 Selected partitions and evolutionary models proposed by Bayesian Inference Criterion through the PhyML algorithm.

PT	Model	Genes	Position of base pairs
1	GTR+I+G	12S 16S	1–946 5238–5915
2	JC+I	28S HH3\2 HH3\3 SAI\1 SAI\2	947–1741 3185–3511\3 3186–3511\3 4264–4660\3 4265–4660\3
3	GTR+I+G	COI\1 CYTB\1	1742–2339 2340–3183\3
4	SYM+I+G	COI\2 CYTB\2 CYTB\3	1743–2339\3 2341–3183\3 2342–3183\3
5	F81	COI\3 RHO\3	1744–2339\3 3949–4263\3
6	HKY+G	HH3\1 RHO\1 TYR\3	3184–3511\3 3947–4263\3 4663–5237\3

8	K80+G	RAG1\1 SAI\3	3512–3946\3 4266–4660\3
9	HKY+I	RAG1\2 RAG1\3 RHO\2 TYR\1 TYR\2	3513–3946\3 3514–3946\3 3948–4263\3 4661–5237\3 4662–5237\3

Note:

Numbers after gene names denote the codon position. PT, partitions.

The best-fit partition scheme and most probable nucleotide evolution model, considering codon partitioning for protein-coding genes, were inferred with PartitionFinder 2.1.1 (Lanfear et al., 2016) via the CIPRES webserver (phylo.org) using Bayesian Inference Criterion and the PhyML algorithm (Guindon et al., 2010). The best-fit partition schemes and evolution models for each partition are shown in Table 1. Phylogenetic relationships were reconstructed using Maximum Likelihood inference in IQ-TREE (Trifinopoulos et al., 2016). Clade support was calculated through 10,000 ultrafast bootstrap approximation replicates with 10,000 maximum iterations, a 0.99 minimum correlation coefficient and 10,000 replicates of the Shimodaira-Hasegawa approximate likelihood ratio. Kimura 2-parameter distance (Kimura, 1980) and uncorrected genetic distance were calculated using the 16S mitochondrial gene through MEGA 6.0 (Tamura et al., 2013).

Morphology. Sex was determined by direct observation of secondary sexual characters (e.g., vocal sac and vocal slits in males) and breeding behavior (advertisement call by males, females surrounding active males). Morphometric measurements longer than 10 mm were taken using a digital caliper (to the nearest 0.1 mm); shorter measurements were taken with a micrometer coupled to a stereoscopic microscope. Measurements follow Lima, Sanchez & Souza (2007) and Barrio-Amorós & Santos (2009): snout-vent length (SVL); head length (HL); interorbital distance (IO); head width (HW); snout length (SL); eye-nostril distance (END); inter-nostril distance (IND); eye length (EL); horizontal tympanum diameter (TYM); forearm length (FAL); upper arm length (UAL); thigh length (LL); tibial length (TL); foot length (FL); hand length from the proximal edge of the palmar tubercle to the tip of finger I (HANDI), finger II (HANDII), finger III (HANDIII), and finger IV (HANDIV); disc width of finger III (WFD); palmar tubercle diameter (DPT); width of thenar tubercle (WTT); width of finger III at proximal phalanx (WFP); width of toe IV disc (WTD). Morphological terminology follows Grant et al. (2006). The description and diagnosis follow Lima, Simões & Kaefer (2014). See Table S2 for morphometric measurements of the type series. Developmental stages of tadpoles (lot INPAH 41351) were determined following Gosner (1960). Terminology, diagnostic features, and measurements follow Altig & McDiarmid (1999) and Schulze, Jansen & Köhler (2015).

Tadpole descriptions follow Schulze, Jansen & Köhler (2015). The following morphometric measurements were taken with a micrometer coupled to a stereoscopic microscope: total length (TL), measured from tail tip to snout tip; body length (BL), measured from snout tip to tail insertion; tail length (TAL), measured from tail tip to its insertion into the body; body width (BW); body height (BH); head width (HWLE); tail muscle width (TMW); maximum tail height (MTH); tail muscle height (TMH); interorbital distance (IOD); inter-nostril distance (IND); eye-nostril distance (END); nostril-snout distance (NSD); eye diameter (ED); vent tube length (VTL); spiracle tube length (STL); and oral disc width (ODW).

Call description. Parameters were measured using Raven 1.5 (Bioacoustics Research Program, 2015). The following parameters were measured: call duration (CD), inter-call interval (ICI), notes per calls (NNC), note duration (ND), inter-note interval (INI), note repetition rate (NRR), call dominant frequency (DF), and first-note dominant frequency (FNDF). We analyzed 1–5 calls for each recorded male and 10 notes and their respective inter-note intervals in each call. Measured notes were equally distributed along each call. Bioacoustic terminology follows Köhler et al. (2017). Spectral parameters were measured by spectrograms set as Window = Blackman; Discrete Fourier Transform = 2,048 samples; 3 dB filter bandwidth = 82 Hz. Graphic representation of the advertisement calls was generated in the R environment (R Core Team, 2016) using the Seewave package 2.0.5 (Sueur, Aubin & Simonis, 2008). Seewave was set up as follows: Hanning window, overlap of 85%, and 256 points of resolution (FFT).

Breeding behavior. Notes regarding breeding behavior were taken from a pair of frogs observed between 07:00 and 08:20 h on 14 February 2019 along state highway AC405 (7°38'54" S, 72°48'59" W, 211 m a.s.l.), municipality of Mâncio Lima, state of Acre, Brazil. A male (INPAH 41347) was observed carrying tadpoles on his back at the same locality later that day.

Interspecific comparisons. The new species inhabits lowland forest in southwestern Brazilian Amazonia near the border with Peru. Hence, we compare the new species with congeners distributed in Brazilian Amazonia south of the Amazon River and in Bolivian and Peruvian Amazonia (Fig. 1): *A. bacurau* Simões, 2016; *A. brunneus* (Cope, 1887), *A. caeruleodactylus* (Lima & Caldwell, 2001); *A. carajas* Simões, Rojas & Lima, 2019; *A. conspicuus* (Morales, 2002); *A. crombiei* (Morales, 2002); *A. femoralis* (Boulenger, 1884), *A. flaviventris* Melo-Sampaio, Souza & Peloso, 2013; *A. fuscillus* (Morales, 2002); *A. gasconi* (Morales, 2002); *A. grillisimilis* Simões et al., 2013; *A. hodli* Simões, Lima & Farias, 2010; *A. magnussoni* Lima, Simões & Kaefer, 2014; *A. masniger* (Morales, 2002); *A. melanolaemus* (Grant & Rodríguez,

2001); *A. nidicola* (Caldwell & Lima, 2003); *A. nunciatus* Moraes, Pavan & Lima, 2019; *A. ornatus* (Morales, 2002); *A. pacaas* Melo-Sampaio et al., 2020; *A. paleovarzensis* Lima et al., 2010; *A. subfolionidificans* (Lima, Sanchez & Souza, 2007); *A. tapajos* Lima, Simões & Kaefer, 2015; *A. tinae* Melo-Sampaio, Oliveira & Prates, 2018; *A. trilineatus* (Boulenger, 1884); and *A. vanzolinius* (Morales, 2002). In addition, we compare the new species to *A. insperatus* (Morales, 2002) and *A. juami* Simões et al., 2018, which have similar calls. In the following description, characters of compared species are presented inside parentheses unless stated otherwise.

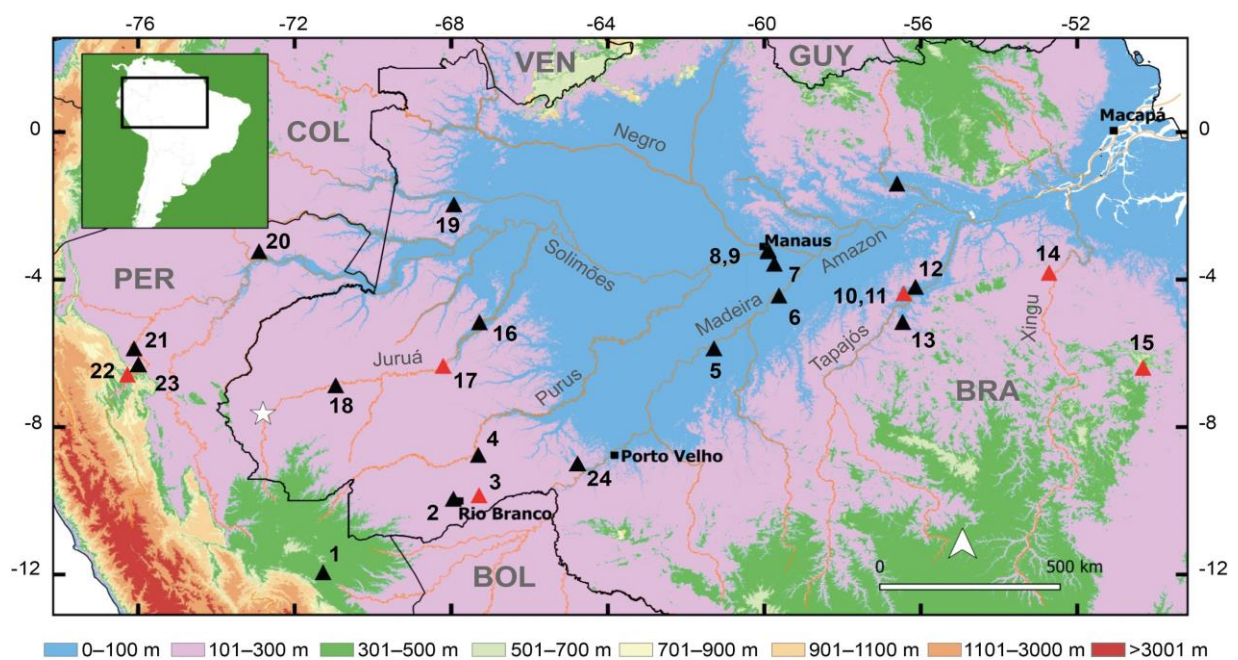


Figure 1 Geographic distribution of the new species and the type localities of other cryptically colored *Allobates* to which it is compared. White star denotes the type locality of the new species. Triangles and numbers denote the type localities of other species: (1) *A. conspicuus*; (2) *A. subfolionidificans*; (3) *A. flaviventris* (4) *A. tinae*; (5) *A. bacurau*; (6) *A. grillisimilis*; (7) *A. nidicola*; (8) *A. paleovarzensis*; (9) *A. caeruleodactylus*; (10) *A. magnussoni*; (11) *A. tapajos*; (12) *A. masniger*; (13) *A. nunciatus*; (14) *A. crombiei*; (15) *A. carajas*; (16) *A. vanzolinius*; (17) *A. gasconi*; (18) *A. fuscillus*; (19) *A. juami*; (20) *A. melanolaemus*; (21) *A. trilineatus*; (22) *A. ornatus*; (23) *A. femoralis*; (24) *A. hodli*. Red triangles denote species with dark marks on the dorsum. Abbreviations: BOL, Bolivia; BRA, Brazil; COL, Colombia; GUY, Guyana; PER, Peru; VEN, Venezuela.

Nomenclatural acts. The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the

nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: urn:lsid:zoobank.org:pub:B411120A-AB6C-4091-81D7-24D52B377BEE. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

RESULTS

Our phylogeny is mostly consistent with previously published phylogenetic hypotheses (Grant et al., 2006; Grant et al., 2017; Melo-Sampaio, Oliveira & Prates, 2018)(Fig. 2). The monophyly of *Allobates* is highly supported (ML = 94%), with *A. olfersioides* emerging as the sister group to all other congeners (ML = 100%). It is followed by *A. undulatus* (ML = 100%) and a clade formed by *A. niputidea* and *A. talamancae* (ML = 100%). Despite the differences between the phylogenetic relationships of some clades recovered in this study and those of previously published phylogenies, most known clades are recovered in our analyses, such as the one composed of *A. nunciatus*, *A. nidicola* and *A. masniger*, and the one that groups *A. subfolionidificans*, *A. conspicuous*, *A. insperatus* and *A. juami* (Fig. 2).

Samples of the new species nest with two other GenBank samples from Cruzeiro do Sul (Acre, Brazil), MF624181 and KY886579, which together form a well-supported clade (ML = 100%). While *A. carajas* is retrieved as sister to the new species, support for this relationship is very weak (ML = 24%). Although they share a similar call structure, the new species is not closely related to *A. crombiei*, *A. amissibilis* or *A. juami*. *A. insperatus* and *A. juami* are recovered as sister species with strong support (ML = 100%), whereas *A. crombiei* is sister to *A. grillisimilis* with weak support (ML = 76%).

The average uncorrected pairwise distance (p-distance) between the new species and its cryptically colored congeners is large: $9.6\% \pm 1.5$ (Table 2). The smallest p-distance is to *A. trilineatus* clade A (6.6%) and the largest is to *A. olfersioides* (13.5%). Distances between the new species and *A. insperatus* (9.7%), *A. juami* (9.5%) and *A. crombiei* (9.9%) are similar to or larger than the average distance between the new species and the entire dataset. See Table 2 for interspecific K2P distances. Average intraspecific genetic distances are small (p-distance and K2P = 0.3%).

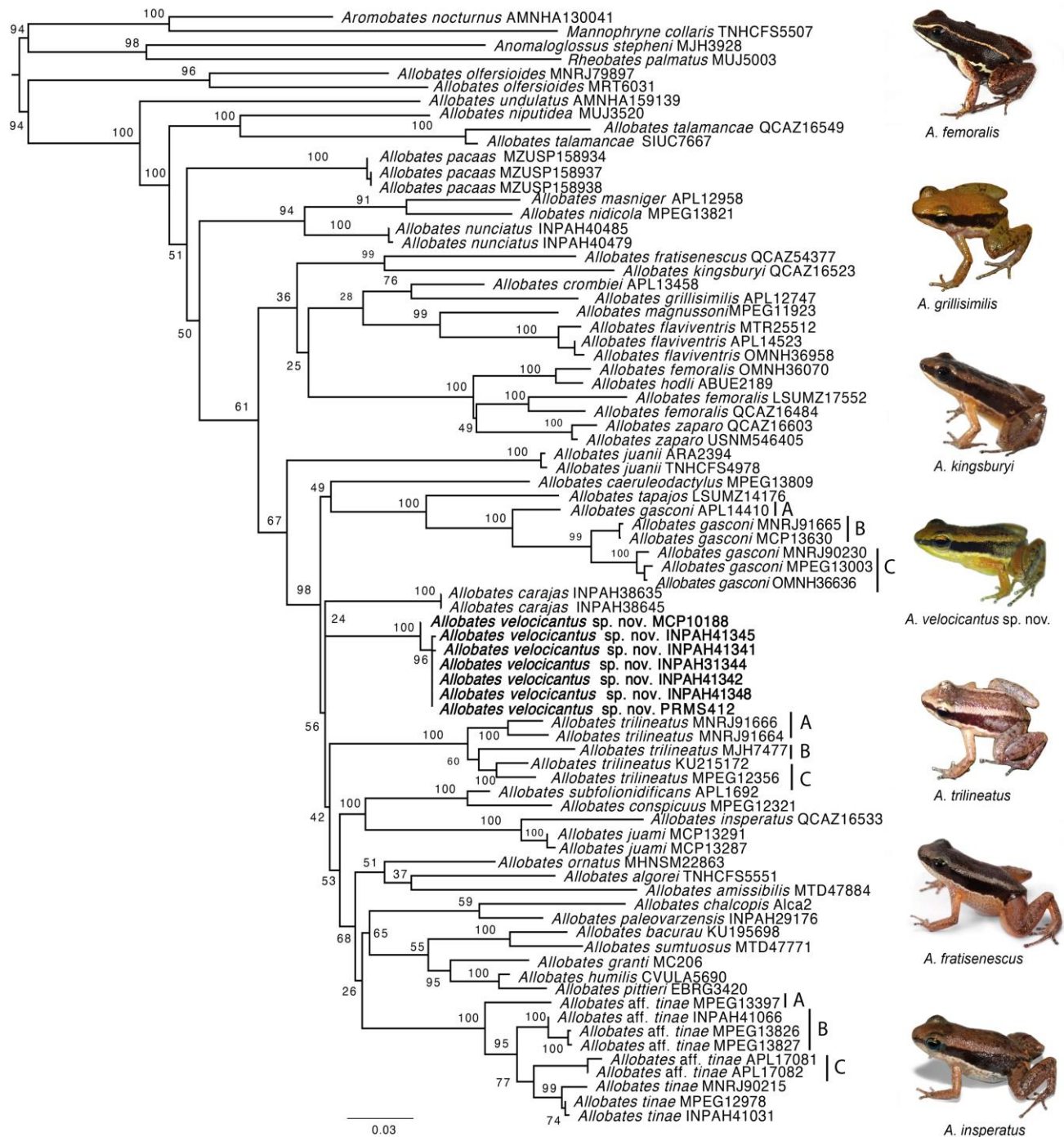


Figure 2 **Phylogeny of the genus *Allobates* reconstructed based on Maximum Likelihood analysis of four mitochondrial genes (12S, 16S, COI, CYTB) and six nuclear genes (28S, HH3, RAG1, RHO, SIA, TYR).** Clade support from 10,000 bootstraps are depicted close to nodes. Photographs by Santiago Ron—www.bioweb.bio (*A. insperatus*, *A. femoralis*, *A. fratisenescus*, *A. trilineatus*, and *A. kingsburyi*), and Jesus R. D. Souza (*A. velocicantus* sp. nov.).

Table 2 **Pairwise interspecific genetic distances between *Allobates velocicantus* sp. nov. and other species of *Allobates* included in the phylogenetic tree.**

Species	<i>p</i>	K2P	Species	<i>p</i>	K2P
<i>Allobates algorei</i>	9.7	10.5	<i>Allobates masniger</i>	10.8	11.8
<i>Allobates amissibilis</i>	8.9	9.6	<i>Allobates nidicola</i>	10.4	11.4
<i>Allobates bacurau</i>	10.0	10.8	<i>Allobates niputidea</i>	11.4	12.6
<i>Allobates caeruleodactylus</i>	7.7	8.2	<i>Allobates nunciatus</i>	9.6	10.4
<i>Allobates carajas</i>	6.8	7.2	<i>Allobates olfersioides</i>	13.5	15.0
<i>Allobates chalcopis</i>	8.2	8.7	<i>Allobates ornatus</i>	9.5	10.3
<i>Allobates conspicuus</i>	9.5	10.3	<i>Allobates pacaas</i>	10.3	11.2
<i>Allobates crombiei</i>	9.9	10.7	<i>Allobates paleovarzensis</i>	7.5	7.9
<i>Allobates flaviventris</i>	10.0	10.8	<i>Allobates pittieri</i>	11.8	13.1
<i>Allobates fratisenescus</i>	9.4	10.1	<i>Allobates subfolionidificans</i>	9.9	10.7
<i>Allobates gasconi</i> A	9.1	9.8	<i>Allobates sumtuosus</i>	8.9	9.5
<i>Allobates gasconi</i> B	9.2	9.9	<i>Allobates talamancae</i>	11.2	12.3
<i>Allobates gasconi</i> C	9.7	10.4	<i>Allobates tapajos</i>	9.8	10.7
<i>Allobates granti</i>	12.1	13.5	<i>Allobates tinae</i>	9.4	10.1
<i>Allobates grillisimilis</i>	9.9	10.8	<i>Allobates aff. tinae</i> A	9.0	9.7
<i>Allobates humilis</i>	10.2	11.1	<i>Allobates aff. tinae</i> B	9.2	9.9
<i>Allobates insperatus</i>	9.7	10.5	<i>Allobates aff. tinae</i> C	8.9	9.5
<i>Allobates juami</i>	9.5	10.3	<i>Allobates trilineatus</i> A	6.8	7.2
<i>Allobates juanii</i>	11.2	12.2	<i>Allobates trilineatus</i> B	6.6	6.9
<i>Allobates kingsburyi</i>	8.8	9.5	<i>Allobates trilineatus</i> C	6.8	7.2
<i>Allobates magnussoni</i>	9.7	10.5	<i>Allobates undulatus</i>	11.9	13.1

Note:

Genetic distances, presented as percentages, were calculated based on a 678-bp fragment of the 16S rRNA gene. Abbreviations: *p*, *p*-distance; K2P, Kimura 2-parameter distance.

TAXONOMY

Allobates velocicantus **sp. nov.**

LSID: urn:lsid:zoobank.org:act:8EAF9324-F176-4A5F-8788-9ACE3E885173.

Allobates sp. CdS MCP10187 (MCP10188) Grant et al. (2017).

Holotype. INPAH 41342 (field number APL 21398, GenBank accession number MT446461), an adult male from along state highway AC405, municipality of Mâncio Lima, state of Acre, Brazil (738°54" W, 7248°59" S, 211 m a.s.l.), collected by J.R.D. Souza and A.P. Lima on 13 January 2019.

Paratopotypes. Eleven specimens: eight males, INPAH 41338–40 (field numbers APL 21391–93), 41341 (field number APL 21397, GenBank accession number MT446462), 41343 (field number APL 21399), 41346–47 (field numbers APL 21402–03), 41348 (field number APL 21404, GenBank accession number MT446458); and three females, INPAH 41344–45 (field numbers APL 21400 (GenBank accession number MT446460) and 21401 (GenBank accession number MT446459)), and 41349 (field number APL 21405); collected by J.R.D. Souza and A.P. Lima on 13 and 14 January 2019. INPAH 413445 is the designated allotype.

Paratype. One adult male, INPAH 41350 (field number APL 21410), from along the road connecting Cruzeiro do Sul to Guajará (734°2" S; 7239°32" W, 181 m a.s.l.), municipality of Cruzeiro do Sul, state of Acre, Brazil, collected by J.R.D. Souza on 15 February 2019.

Etymology. The specific epithet is derived from the Latin words *velox* (= fast) and *cantus* (= singing), in reference to the high note-repetition rate of the advertisement call of the new species. Proposed standard English name: fast singer frog. Proposed standard Spanish name: sapito del canto veloz. Proposed standard Portuguese name: sapinho do canto acelerado.

Phylogenetic placement. The new species is assigned to the genus *Allobates* based on its phylogenetic position presented in the present study and in Grant et al. (2017).

Diagnosis. *Allobates velocicantus* sp. nov. is characterized by (1) small size, SVL 14.9–16.2 mm in males and 16.0–17.4 mm in females; (2) dorsal color pattern predominantly light brown, with no dark patches and marks; (3) dorsum granular; (4) dark brown lateral stripe from the tip of the snout to the groin; (5) light dorsolateral stripe absent or inconspicuous in living specimens but present in preserved specimens; (6) light but incomplete ventrolateral stripe; (7)

oblique lateral line diffuse; (8) snout slightly rounded in dorsal view; (9) tympanum inconspicuous; (10) paired dorsal digital scutes; (11) supernumerary tubercles absent; (12) distal tubercle absent on finger IV; (13) discs moderately expanded on fingers I–IV; (14) finger I slightly larger than finger II; (15) finger III of males with similar width along phalanges; (16) dermal lateral fringes and webbing absent on fingers; (17) metacarpal ridge absent; (18) carpal pad absent; (19) excrescences on thumbs absent; (20) dark gland on arm absent; (21) basal webbing present between toes III and IV; (22) metatarsal tubercle present; (23) light “half-moon” shaped paracloacal mark; (24) superior eyelids dark brown; (25) iris metallic bronze; (26) pupil large, black, horizontal semielliptical; (27) transverse bars on thigh absent; (28) dark brown spots on tibia; and (29) advertisement calls formed by 66–138 pulsed notes (emitted in multiple exhalations) with a dominant frequency of 5,512–6,158 Hz.

Description of the holotype. Adult male, INPAH 41342 (field number APL 21398), SVL = 15.3 mm (Figs. 3A–3C, 4A, 5A and 6A–6C). Measurements of the holotype are presented in Table 3. Head 23% wider than long. Head width and length equal 35% and 30% of SVL, respectively. Eye diameter larger than distance from eye to nostril ($EN/EL = 0.59$). Eye diameter 50% of head length. Interorbital distance 81% of head width. Tympanum inconspicuous to the naked eye. Snout rounded in dorsal and lateral views. Nares located laterally at the tip of the snout. Canthus rostralis barely defined, slightly straight in dorsal view and rounded in lateral view; loreal region slightly concave. Maxillary teeth absent. Median lingual process absent. Tongue three times longer than wide (3.6 mm vs. 1.2 mm). Vocal sac single and subgular. Lateral vocal slits present at the height of the jaw angle.

Palmar tubercle rounded, conspicuous, 0.6 mm wide. Thenar tubercle elliptical, conspicuous, 0.25 mm wide. Thenar tubercle width 42% of palmar tubercle diameter. Distal subarticular tubercle on finger III laterally positioned, tiny and barely noticeable. Distal subarticular tubercle on finger IV absent. Additional subarticular tubercles on fingers III and IV small and rounded, not exceeding phalangeal width. Subarticular tubercles on fingers I and II oval, protuberant and as wide as phalanges, corresponding to 2 and 1.4 times the width of the thenar tubercle, respectively. Supernumerary tubercles absent. Finger fringes and hand webbing absent. Tip of finger IV does not reach the distal subarticular tubercle of finger III when juxtaposed. Preaxial expansion of finger III, from the base to the tip of the finger. Finger I slightly longer than finger II. Relative length of fingers: $IV < II < I < III$. Discs of fingers II, III and IV slightly wider than the third phalange; disc of finger I of similar width to the distal phalange. Paired digital scutes present.

Thigh and tibia lengths similar, each 48% of body length. Foot length 88% of tibia length. Tarsal keel conspicuous, curved, straightening until almost forming a line towards, but not reaching, the inner metatarsal tubercle. Inner metatarsal tubercle protuberant and elliptical. Outer metatarsal tubercle small and rounded, protuberant, four times the diameter of the inner metatarsal tubercle. Metatarsal fold absent. Fringes on toes absent. Basal webbing present only between toes III and IV. Subarticular tubercle on toe I oval and protuberant. Subarticular tubercles on toes II–V rounded and barely evident; one subarticular tubercle on toe II, two on toes III and V, and three on toe IV. Disc of toe I same width as phalanx, tip rounded. Discs of toes II–V expanded laterally, tips rounded. Paired digital scutes present.

Skin on dorsum granular. Skin on throat, chest, belly and ventral surfaces of limbs smooth.

In preservative, dorsum of the body cream, with elevated concentration of dark granules and melanophores extending from the interorbital region to the urostyle (Fig. 3A). Dorsolateral stripe present. Dorsal region of arms, forearms, thighs and paracloacal marks cream (Fig. 3A). Tibia with dark brown patches of varied sizes. A dark brown lateral stripe surrounds the whole body, but it is narrower in the loreal region and of constant width in the lateral part of the body. Anterior and posterior regions of the thigh dark brown. Ventrolateral stripe present but barely noticeable and composed of irregular small whitish patches (Fig. 3B). Belly, chest, forearms and throat cream; melanophores grouped in the anterior portion of the throat. Ventral surface of arms, thighs and tibia cream, with melanophores present in the distal region of the thighs and peripheral regions of the tibia (Fig. 3C). Ventral surfaces of the hands and feet dark brown (Figs. 4A and 5B).

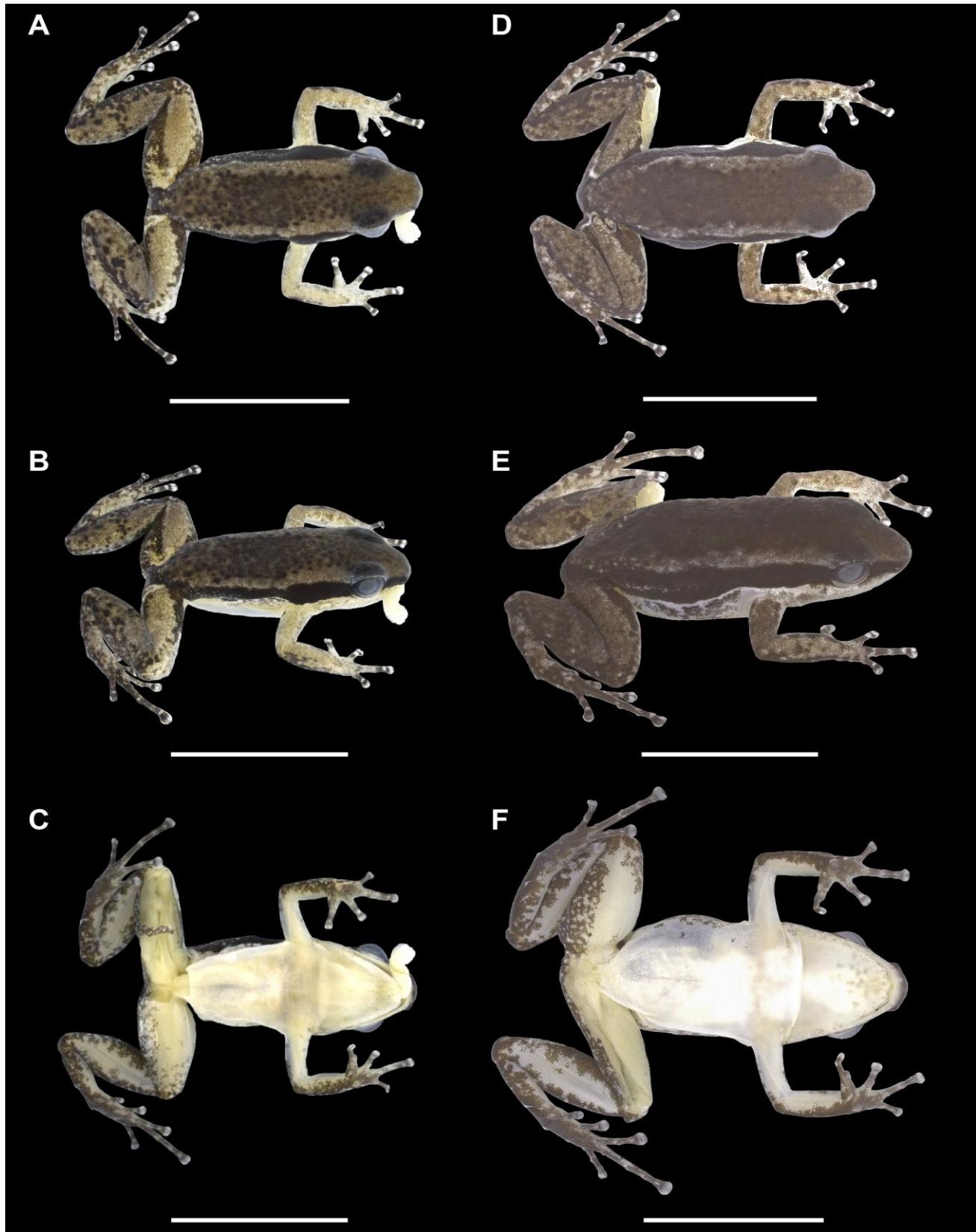


Figure 3 Dorsal, dorsolateral and ventral views of the holotype (INPAH 41342) and allotype (INPAH 41345) of *Allobates velocicantus* sp. nov. (A–C) Holotype. (D–F) Allotype. Scale bar: 10 mm. Photographs by Jeni Lima Magnusson.

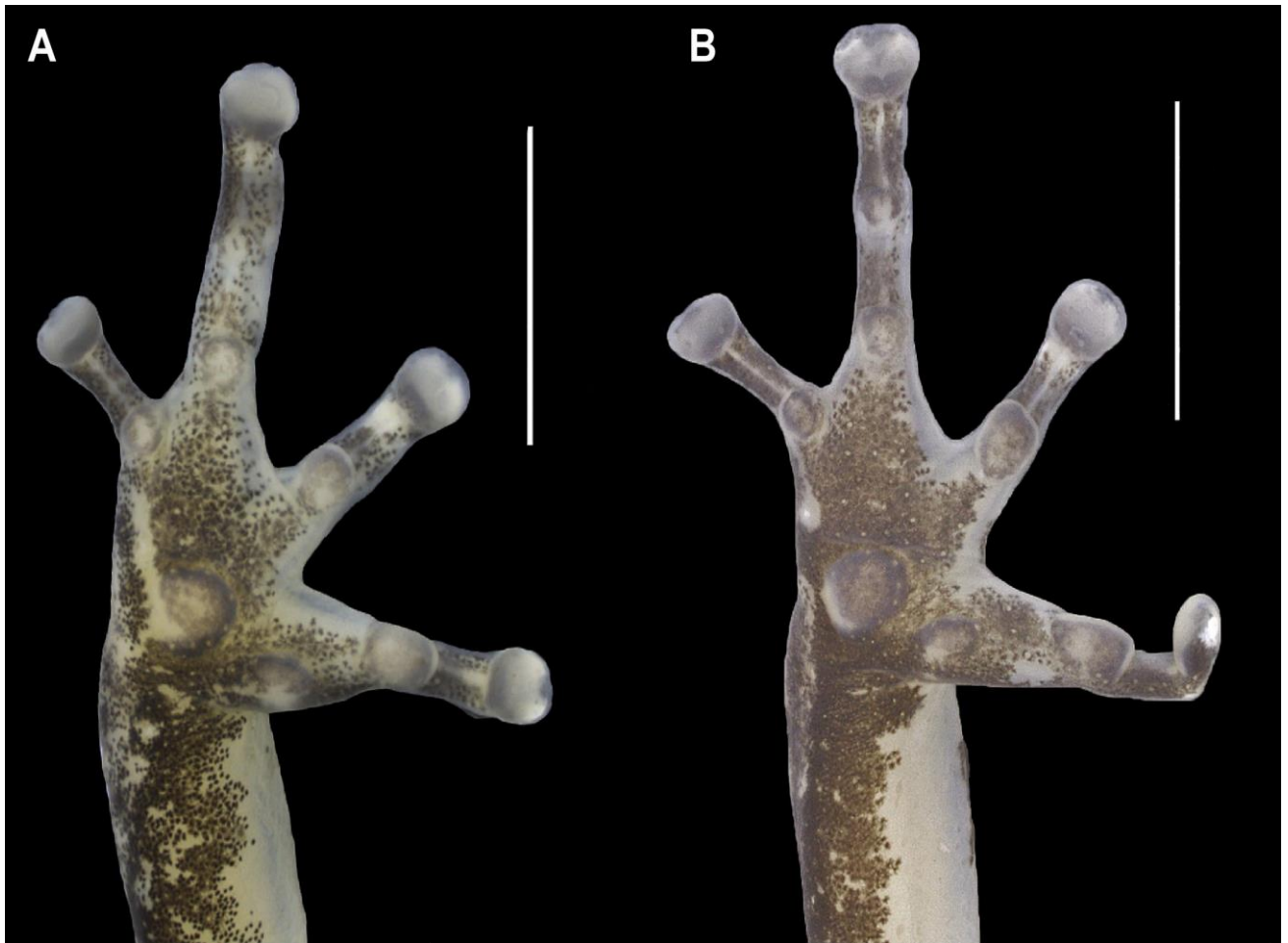


Figure 4 **Ventral view of the right hand of the holotype (INPAH 41342) and allotype (INPAH 41345) of *Allobates velocicantus* sp. nov.** (A) Holotype. (B) Allotype. Scale bar: 2 mm. Photographs by Jeni Lima Magnusson.

Variation. On average, females are slightly larger (SVL $16.7 \text{ mm} \pm 0.7$, 16.0–17.4; $n = 3$) than males (SVL $15.4 \text{ mm} \pm 0.3$, 14.9–16.2; $n = 10$) (Table 3). However, the small number of females impedes statistical tests to verify sexual dimorphism. Females differ from males by having thinner phalanges on finger III. In both males and females, the central region of the dorsum varies in the concentration of dark granules and melanophores: five specimens show a dense concentration of dark granules (thus resembling the holotype), whereas in six other specimens such a concentration is less evident. The dorsolateral stripe varies in width and visibility; in both males and females, it is absent or barely noticeable in life but more evident in preservative. The ventrolateral stripe is conspicuous in living specimens but almost invisible to the naked eye in preserved specimens, being formed by whitish patches with no melanophores.



Figure 5 **Ventral view of the right foot of the holotype (INPAH 41342) and allotype (INPAH 41345) of *Allobates velocicantus* sp. nov.** (A) Holotype. (B) Allotype. Scale bar: 5 mm. Photographs by Jeni Lima Magnusson.

The anterior region of the throat has a variable amount of melanophores in both males and females. The tibia shows dark patches, which form a transverse line visible in five specimens but not in six others. The tarsal keel varies in size, shape and length; it reaches the external edge of the inner metatarsal tubercle in only one specimen, INPAH 41341.

In life, dorsum brown with dark brown granules concentrated medially. Dorsal surface of arms and forearms orange brown. Thighs light brown dorsally but dark brown at anterior and posterior ends. Supraorbital region light brown. Dark brown lateral stripe of constant width,

narrower in the loreal region and wider in the flanks; small whitish dots inside the brown lateral line at the inguinal region. Dorsolateral stripe, when present, light brown. An irregular ventrolateral stripe is iridescent white. It is formed by dots in some specimens; in others, it is well defined and complete or incomplete (Figs. 6B, 6E, 6H and 6K). Ventrolateral surfaces of thighs and arms, as well as the flanks, are translucent beige. Belly white in males and females; chest white in females and translucent in males. Throat of females translucent with the edges of the jaw yellowish. In males, the vocal sac, when deflated, is whitish centrally and yellow peripherally. When inflated, it is mostly white with translucent yellow edges. Iris metallic bronze; pupils black, horizontal semielliptical.

Comparisons with other species. *Allobates velocicantus* sp. nov. easily differs from *A. femoralis* and *A. hodli* by the absence of bright yellow, orange or red marks on thighs (present). Cryptically colored species of *Allobates* show either of two striking dorsal color patterns, one with and the other without large dark patches or marks, a characteristic that helps to quickly differentiate species. *Allobates velocicantus* sp. nov. lacks a dark hourglass-shaped mark on the dorsum and is thus easily distinguished from *A. brunneus*, *A. carajas*, *A. crombiei*, *A. flaviventris*, *A. gasconi*, *A. magnussoni*, *A. ornatus*, *A. pacaas*, *A. tapajos*, and *A. trilineatus* (all of which have dark patches or marks on the dorsum with rhomboid-, diamond-, hourglass- or triangle-shaped patterns).

Among *Allobates* species that lack dark patches or marks on the dorsum, throat color is a reliable characteristic for differentiating species. Males of *Allobates velocicantus* sp. nov. has a white throat with yellow edges, which distinguishes it from males of *A. bacurau* (light to dark gray), *A. fuscillus* (dark to solid dark), *A. juami* (pinkish to translucent), *A. masniger* (dark gray), *A. melanolaemus* (black), *A. nidicola* (dark gray), *A. nunciatus* (violaceous), *A. paleovarzensis* (grayish-violet) and *A. vanzolinius* (light to dark gray).

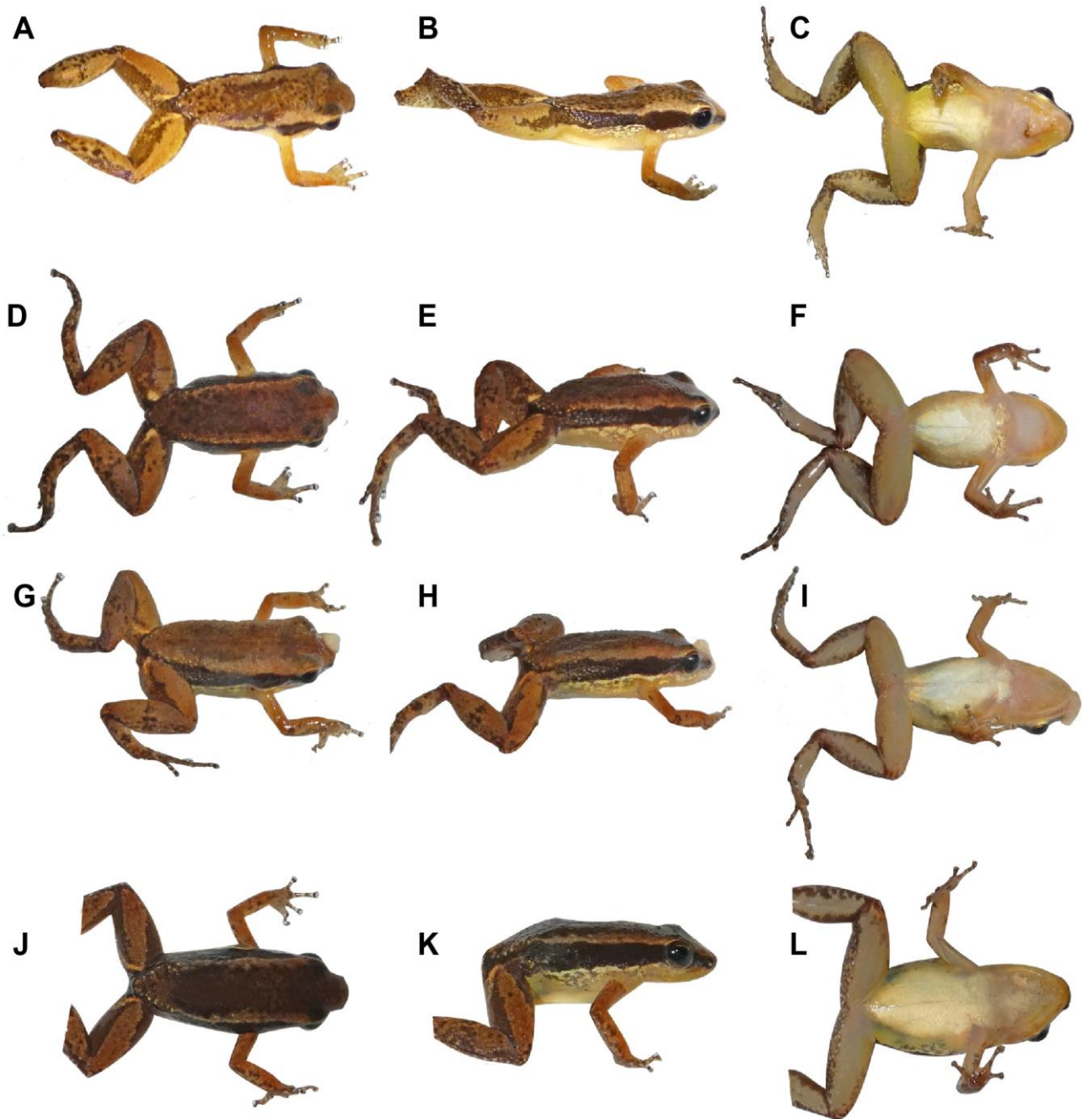


Figure 6 **Coloration in life of adult *Allobates velocicantus* sp. nov.** (A–C) Holotype, male, INPAH 41342, SVL = 15.3 mm. (D– F) Male, INPAH 41341, SVL = 15.4 mm. (G–I) Female, INPAH 41344, SVL = 16.6 mm. (J–L) Female, INPAH 41345, SVL = 17.4 mm. Photographs by Albertina Pimentel Lima.

Table 3 **Morphometric measurements (in millimeters) of *Allobates velocicantus* sp. nov.**

Characters	Holotype	Males (n = 10)	Females (n = 3)
SVL	15.3	15.4 ± 0.3 (14.9–16.2)	16.7 ± 0.7 (16.0–17.4)
IO	4.3	4.3 ± 0.2 (4.0–4.6)	4.6 ± 0.2 (4.4–4.8)
HW	5.3	5.2 ± 0.2 (5.0–5.5)	5.5 ± 0.2 (5.4–5.7)
HL	4.6	4.9 ± 0.2 (4.5–5.1)	5.2 ± 0.1 (5.1–5.3)
IN	2.3	2.2 ± 0.1 (2.2–2.4)	2.4 ± 0.1 (2.5–2.5)
LL	7.3	7.1 ± 0.2 (6.7–7.5)	7.4 ± 0.5 (7.1–8.0)
TL	7.4	7.4 ± 0.3 (6.6–7.7)	7.7 ± 0.4 (7.4–8.2)
FL	6.5	6.8 ± 0.3 (6.3–7.2)	7.1 ± 0.1 (7.0–7.2)
UAL	4.0	4.1 ± 0.2 (3.7–4.4)	4.4 ± 0.2 (4.2–4.6)
FAL	3.4	3.2 ± 0.2 (2.9–3.6)	3.3 ± 0.2 (3.2–3.5)
HANDI	3.0	3.2 ± 0.2 (2.9–3.7)	3.5 ± 0.4 (3.1–3.9)
HANDII	2.9	2.9 ± 0.2 (2.8–3.4)	3.2 ± 0.2 (3.0–3.3)
HANDIII	4.1	4.0 ± 0.2 (3.8–4.3)	4.2 ± 0.3 (4.1–4.5)
HANDIV	2.6	2.5 ± 0.2 (2.3–3.0)	2.7 ± 0.1 (2.6–2.8)
WFD	0.7	0.6 ± 0.1 (0.6–0.8)	0.7 ± 0.1 (0.6–0.8)
WPF	0.5	0.5 ± 0.1 (0.4–0.6)	0.4 ± 0.1 (0.4–0.5)
DPT	0.6	0.6 ± 0.0 (0.6–0.6)	0.7 ± 0.1 (0.6–0.8)

WTT	0.3	0.3 ± 0.1 (0.2–0.4)	0.3 ± 0.0 (0.3–0.4)
WTD	0.7	0.8 ± 0.1 (0.6–0.9)	0.8 ± 0.0 (0.8–0.9)
TYM	1.1	1.1 ± 0.1 (1.0–1.2)	1.0 ± 0.1 (1.0–1.1)
EL	2.3	2.3 ± 0.1 (2.2–2.5)	2.4 ± 0.1 (2.4–2.5)
EN	1.4	1.6 ± 0.1 (1.5–1.7)	1.7 ± 0.0 (1.7–1.7)
SL	2.2	2.5 ± 0.1 (2.4–2.8)	2.8 ± 0.1 (2.8–2.9)
FL/TL	0.9	0.9 ± 0.1 (0.8–1.1)	0.9 ± 0.0 (0.9–1.0)
EN/EL	0.6	0.7 ± 0.0 (0.6–0.7)	0.7 ± 0.0 (0.7–0.7)
HL/SVL	0.3	0.3 ± 0.0 (0.3–0.3)	0.3 ± 0.0 (0.3–0.3)
HW/SVL	0.4	0.3 ± 0.0 (0.3–0.4)	0.3 ± 0.0 (0.3–0.3)
HL/HW	0.9	0.9 ± 0.1 (0.9–1.0)	1.0 ± 0.0 (0.9–1.0)
FAL/SVL	0.2	0.2 ± 0.0 (0.2–0.2)	0.2 ± 0.2 (0.2–0.2)
TL/SVL	0.5	0.5 ± 0.0 (0.4–0.5)	0.5 ± 0.0 (0.5–0.5)

Note:

Values are presented as the mean $\pm$ standard deviation, with the range in parentheses.

Table 4 Temporal and spectral characterization of advertisement calls of *Allobates velocicantus* sp. nov.

Voucher	SVL (mm)	°C	AC	CD (s)	ICI (s)	NNC	ND (ms)	INI (ms)	DF (Hz)	FNDF (Hz)	RR
INPAH 41338	16.2	26.0	5	2.1	57.3	121	8.9	12.0	5,551	5,108	56.8
INPAH 41339	15.5	24.7	3	2.4	48.9	130	9.6	13.0	5,756	4,809	53.9
INPAH 41341	15.4	24.7	3	2.2	78.5	119	9.6	10.0	5,835	4,888	53.8
INPAH 41342	15.3	26.0	2	2.6	45.9	126	8.0	12.0	5,871	5,369	49.0
INPAH 41343	15.5	26.0	1	2.6	71.9	133	9.4	11.0	5,900	5,060	51.5
INPAH 41346	15.6	24.7	4	2.4	53.2	123	8.2	12.0	5,922	4,802	50.4
INPAH 41348	14.9	27.4	2	2.2	31.8	123	7.1	12.0	5,965	5,426	55.6
INPAH 41350	15.5	26.0	4	2.8	66.7	107	9.0	12.0	6,094	5,828	38.4
Mean	15.5	25.7	3	2.4	58.3	121	8.7	13.0	5,831	5,133	51.2
SD	0.4	0.9	1	0.3	16.6	15	1.9	2.0	168	386	5.8
Min	14.9	24.7	1	1.9	31.8	66	5.0	10.0	5,512	4,263	38.4
Max	16.2	27.4	5	2.9	107.2	138	13.0	18.0	6,158	5,900	56.8

Note:

Values for mean, standard deviation (SD), minimum (Min) and maximum (Max) are based on all analyzed calls. Additional abbreviations: AC, number of analyzed calls; CD, call duration; DF, dominant frequency; FNDF, dominant frequency of the first note; ICI, inter-call interval; INI, inter-note interval; ND, note duration; NNC, number of notes per call; RR, repetition rate.

Allobates velocicantus sp. nov. could be mistaken for *A. caeruleodactylus*, *A. conspicuus*, *A. grillisimilis*, *A. subfolionidificans* or *A. tinae*, which have similar ventral color patterns in preservative and adult males of similar size (SVL). However, *Allobates velocicantus* sp. nov. differs from *A. caeruleodactylus* by lacking basal webbing between fingers II–III and by scutes of fingers white (basal webbing between fingers II–III present, scutes of fingers blue); from *A. conspicuus* by having finger I slightly longer than finger II and by lacking both fringes on toes and transverse bars on thighs (finger I considerably longer than finger II, fringes on toes present and transverse bars on thighs present); from *A. grillisimilis* by the absence of a conspicuous transverse bar on legs and arms, finger III with phalanges of uniform width, edges of the throat yellow with center whitish (varying number of transverse bars on legs and arms present, finger III with phalanges of non-uniform width, and throat translucent white); and from *A. subfolionidificans* by having a white belly and a whitish throat with yellow edges, a ventrolateral stripe, and by lacking conspicuous transverse bars on the thighs (belly and throat opaque white, ventrolateral stripe absent, and transverse bars on thighs present).

Advertisement call. Spectral and temporal parameters of eight recorded males are presented in Table 4. Advertisement calls of *Allobates velocicantus* sp. nov. (Fig. 7) comprise trills of 66–138 (121 ± 15) pulsed notes emitted at regular intervals, with a call duration of 1.87–2.89 s (2.49 ± 0.22). Inter-call intervals vary from 31.8 to 107.2 s (58.3 ± 16.6). Notes are emitted through multiple exhalations, are always composed of two pulses and have an average note duration of $8.7 \text{ ms} \pm 1.9$ (5–13). Inter-note interval has an average duration of $13 \text{ ms} \pm 2$ (10–18). The average dominant frequency is $5,831 \text{ Hz} \pm 168$ (5,512–6,158). The dominant frequency of the first note of each call is considerably lower than that of other notes (Fig. 7B), varying from 4,263 to 5,900 Hz ($5,133 \text{ Hz} \pm 386$). Each call is considerably lower than that of other notes (Fig. 7B), varying from 4,263 to 5,900 Hz ($5,133 \text{ Hz} \pm 386$).

Bioacoustic comparisons. *Allobates velocicantus* is easily differentiated from *A. caeruleodactylus*, *A. magnussoni*, *A. masniger*, *A. nidicola* and *A. subfolionidificans* by emitting trills with 66–138 pulsed notes (advertisement call composed by a single note emitted continuously or irregularly in all these species: Lima & Caldwell, 2001; Caldwell & Lima, 2003; Lima, Simões & Kaefer, 2014; Lima, Sanchez & Souza, 2007; Tsuji-Nishikido et al., 2012).

The advertisement call of *A. velocicantus* sp. nov. has only one type of temporal arrangement of notes (trills), which distinguishes it from the calls of *A. brunneus*, *A. carajas*, *A. flaviventris* and *A. tapajos* (each of these species has two types of temporal arrangement of notes: notes emitted continuously, or in trills of unpulsed notes).

In addition, by emitting trills with 66–138 pulsed notes, *A. velocicantus* sp. nov. differs from *A. brunneus* (trills of 6–11 notes; Lima, Caldwell & Strüssmann, 2009), *A. carajas* (trills of 4–22 notes; Simões, Rojas & Lima, 2019), *A. flaviventris* (trills of 2–10 notes; Melo-Sampaio, Souza & Peloso, 2013) and *A. tapajos* (trills of 10–14 notes; Lima, Simões & Kaefer, 2015).

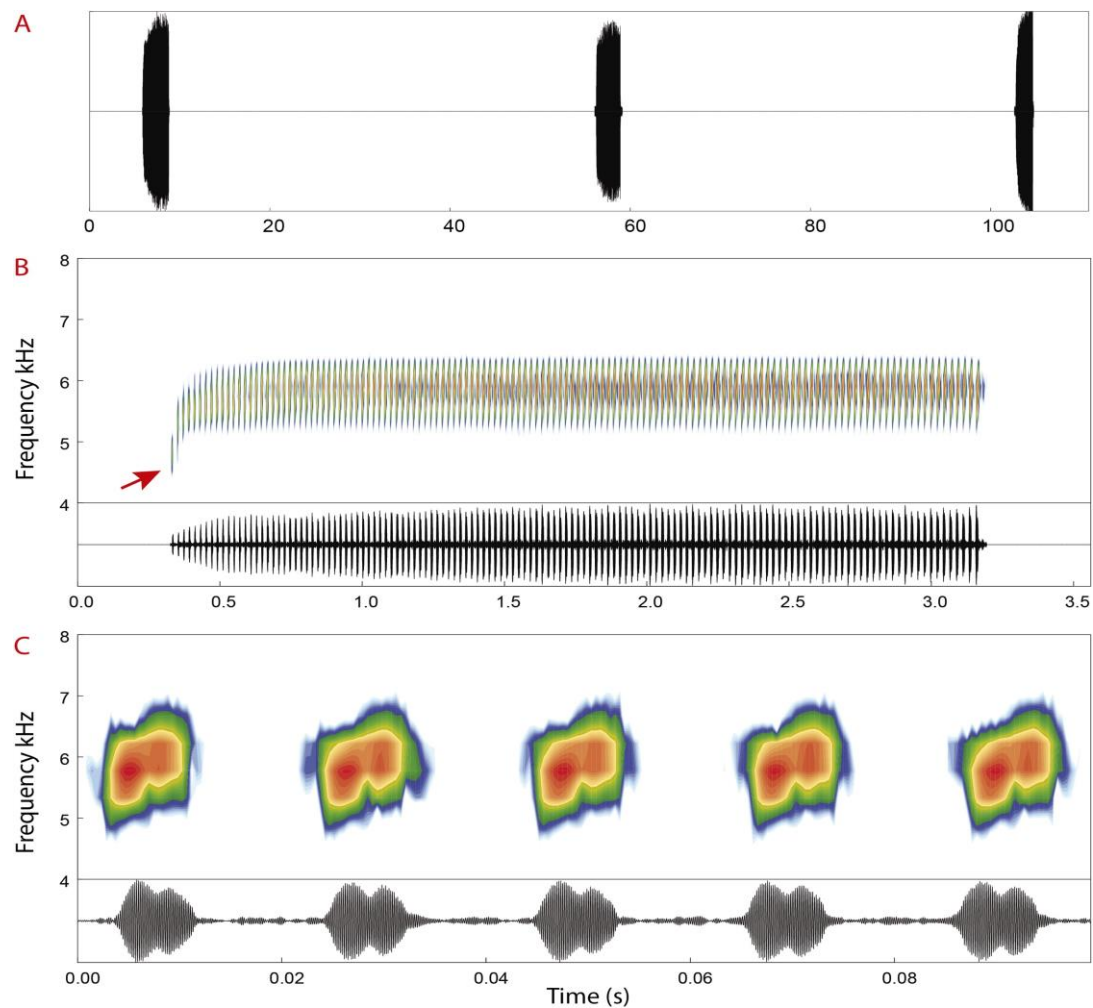


Figure 7 **Advertisement call of *Allobates velocicantus* sp. nov.** (A) Oscillogram showing three calls. (B) Spectrogram and oscillogram of a call composed of 137 pulsed notes. (C) Detailed view of five pulsed notes of the call depicted in (B). The red arrow denotes the first note, which has a lower dominant frequency (4,737 Hz) than later notes. Recorded male: INPAH 41346. Air temperature: 24.7 C.

Since the advertisement call of *A. velocicantus* sp. nov. has a maximum duration of 2.9 s

and is composed of trills with as many as 138 notes and inter-note intervals of 10–18 ms, the new species can be differentiated from *A. bacurau* (maximum call duration 11.1 s, maximum notes 81, inter-note interval 71–129 ms; Simões, 2016), *A. crombiei* (maximum call duration 4.5 s, maximum notes 59, inter-note interval 45–69 ms; Lima, Erdtmann & Amézquita, 2012), *A. femoralis* (maximum call duration 0.7 s, maximum notes 6, inter-note interval 51–140 ms; Moraes, Pavan & Lima, 2019), *A. granti* (maximum call duration 4.5 s, maximum notes 17, inter-note interval 110–250 ms; Kok et al., 2006), *A. hodli* (maximum call duration 0.2 s, maximum notes 2, inter-note interval 62–99 ms; Simões, Lima & Farias, 2010), *A. insperatus* (maximum call duration 3.7 s, maximum notes 45, inter-note interval 42–85 ms; Simões et al., 2018), *A. juami* (maximum call duration 5.1 s, maximum notes 73, inter-note interval 20–50 ms; Simões et al., 2018), *A. nunciatus* (maximum call duration 357 ms, maximum notes 10, inter-note interval 29–85 ms; Moraes, Pavan & Lima, 2019), *A. paleovarzensis* (maximum call duration 3.0 s, maximum notes 21, inter-note interval 65–266 ms; Lima et al., 2010), *A. tinae* (maximum call duration 3.7 s, maximum notes 13, inter-note interval 100–410 ms; Lima, Ferrão & Silva, in press) and *A. trilineatus* (maximum call duration 1.6 s, maximum notes 13, inter-note interval 71–88 ms; Grant & Rodríguez, 2001).

Color of freshly laid eggs and larvae description. Freshly laid eggs of *A. velocicantus* sp. nov. have dark gray animal pole covering approximately two thirds of the animal hemisphere. The dark gray animal pole shows a well-delimited edge above the whitish equatorial portion. The vegetal pole is completely whitish. Eggs are deposited in a cloudy jelly.

Morphometric measurements of tadpoles are presented in Table 5. See Figs. 8 and 9 for body form and oral disc. Descriptions of quantitative characters are based on tadpoles at Gosner stage 34 (n = 5). Body ovoid, rounded anteriorly and posteriorly in dorsal view, flattened in lateral view (BH/BW = 0.7–0.8). Body length and tail length 30.7–33.0% and 66.9–69.3% of TL, respectively; HWLE 81.5–90.9% of BW; snout rounded in lateral and dorsal views; END 60.0–76.5% of ED; eyes dorsal and directed laterally; IOD 54.8–58.6% of HWLE. Small nares located dorsolaterally and directed anterolaterally, visible in dorsal and lateral views; internarial distance 40.0–45.2% of HWLE. Fleishy ring on inner margin of nostrils rounded, not ornamented. Spiracle single, sinistral, tubular, 0.6–0.9 mm long, attached to body ventrolaterally, slightly below mid-body length. Gut coiled and visible through the skin to the naked eye, with its axis directed to the left side of the body. Vent tube dextral, 1.4–2.2 mm long. Maximum tail height 2.5–2.9 mm. Dorsal fin emerges after 2.0–2.5 mm from the limit

between tail and body; dorsal edge shallow and straight anteriorly (along approximately 10% of its length) but deeper posteriorly, reaching maximum height at two thirds of the tail length.

Table 5 Morphometric measurements (in millimeters) of 14 tadpoles of *Allobates velocicantus* sp. nov., Gosner stages 27–37, collected in the municipality of Mâncio Lima, state of Acre, Brazil.

	Stage 27 (n = 1)	Stage 31 (n = 1)	Stage 32 (n = 3)	Stage 33 (n = 2)	Stage 34 (n = 5)	Stage 36 (n = 1)	Stage 37 (n = 1)
TL	13.6	16	16.5 ± 0.64 (15.8–16.9)	17.5–17.7	17.2 ± 1.07 (15.7–18.5)	19.3	19.6
BL	4.5	5.2	5.4 ± 0.20 (5.1–5.5)	5.8–6.0	5.6 ± 0.3 (5.2–6.1)	6.3	6.6
TAL	9.1	10.8	11.1 ± 0.44 (10.7–11.5)	11.5–11.9	11.6 ± 0.81 (10.5–12.4)	13	13
BW	2.9	3.5	3.4 ± 0.40 (3–3.7)	3.6–3.7	3.4 ± 0.24 (3.2–3.8)	3.7	4.1
BH	1.9	2.6	2.4 ± 0.40 (2–2.7)	2.7–2.9	2.5 ± 0.34 (2.3–3.1)	2.8	3.1
HWLE	2.3	3	2.8 ± 0.10 (2.7–2.9)	2.8–3.2	2.94 ± 0.11 (2.8–3.1)	3.2	3.2
TMW	1.1	1.4	1.47 ± 0.12 (1.4–1.6)	1.7–1.7	1.7 ± 0.09 (1.6–1.8)	1.5	1.9
MTH	2	2.4	2.5 ± 0.10 (2.4–2.5)	2.5–2.7	2.7 ± 0.15 (2.5–2.9)	2.9	2.8
TMH	1.1	1.5	1.5 ± 0.10 (1.4–1.6)	1.7–1.7	1.7 ± 0.08 (1.6–1.8)	1.7	1.8
IOD	1.3	1.5	1.6 ± 0.10 (1.5–1.6)	1.6–1.7	1.7 ± 0.05 (1.6–1.7)	1.7	1.9
IND	0.9	1.3	1.2 ± 0.10 (1.1–1.3)	1.4–1.4	1.3 ± 0.07 (1.2–1.4)	1.5	1.6
END	0.5	0.5	0.6 ± 0.0 (0.6–0.6)	0.7–0.7	0.6 ± 0.04 (0.6–0.7)	0.55	0.75
NSD	0.4	0.3	0.4 ± 0.03 (0.4–0.5)	0.5–0.5	0.4 ± 0.09 (0.3–0.5)	0.3	0.45
ED	0.8	0.85	0.8 ± 0.10 (0.8–0.9)	1.0–1.0	0.9 ± 0.08 (0.9–1.0)	0.95	1.1
SS	2.7	3	3 ± 0.04 (2.7–3.4)	3.6–3.8	3.4 ± 0.32 (3.1–3.9)	3.1	3.9
VTL	1.1	1.32	1.5 ± 0.08 (1.4–1.6)	1.6–1.9	1.7 ± 0.28 (1.4–2.16)	2	1.72
STL	0.3	0.5	0.7 ± 0.13 (0.6–0.9)	0.8–0.8	0.7 ± 0.11 (0.60–0.85)	0.6	0.75

ODW	1.18	1.46	1.39 ± 0.05 (1.34–1.44)	1.40–1.48	1.47 ± 0.08 (1.38–1.58)	1.6	1.7
PL	0.4	0.44	0.40 ± 0.0 (0.40–0.40)	0.30–0.50	0.40 ± 0.1 (0.28–0.54)	0.58	0.6
AL	0.5	0.5	0.50 ± 0.05 (0.40–0.50)	0.50–0.50	0.46 ± 0.02 (0.44–0.48)	0.5	0.54
GAP	0.4	0.4	0.40 ± 0.0 (0.40–0.40)	0.40–0.40	0.39 ± 0.12 (0.18–0.48)	0.38	0.58
A1	0.9	1	0.90 ± 0.0 (0.90–0.90)	1.04–1.04	1.0 ± 0.23 (0.74–1.30)	NA	NA
A2	0.7	1.1	1.0 ± 0.0 (1.0–1.0)	1.1–1.2	0.94 ± 0.37 (0.28–1.18)	1.16	1.14
P1	0.8	1.04	1.0 ± 0.03 (0.9–1.0)	0.9–1.1	1.02 ± 0.07(0.96–1.12)	1.12	1.28
P2	0.7	0.94	0.9 ± 0.03 (0.8–0.9)	0.8–1.1	0.94 ± 0.07 (0.88–1.06)	0.96	1.2
P3	0.5	0.62	0.46 ± 0.13 (0.34–0.60)	0.62–0.64	0.46 ± 0.08 (0.36–0.56)	0.4	0.68
UJW	NA	0.12	0.11± 0.01 (0.10–0.12)	0.10–0.12	0.11 ± 0.01 (0.10–0.12)	0.12	0.18
UJL	0.7	0.66	0.70 ± 0.0 (0.7–0.7)	0.7–0.7	0.75 ± 0.03 (0.72–0.78)	0.82	0.8

Note:

For stages with more than two tadpoles, values represent the mean ± standard deviation (minimum–maximum). For stages with only two tadpoles, values represent the minimum and maximum. Abbreviations: n, sampling size; NA, not measured. See the main text for abbreviations of morphometric measurements.

Dorsal fin slightly deeper than ventral fin. Ventral fin does not exceed body height. Tail tip slightly acuminate. Caudal musculature in dorsal view attains 47.4–51.5% of body width. In lateral view it reaches 54.8–75.0% body height. Oral disc located anteroventrally, emarginated laterally (Fig. 8); width of oral disc 1.4–1.6 mm, corresponding to approximately 41.6–45.5% of body width at the level of spiracle. Anterior labium with 3–4 short, pyramidal, rounded papillae distributed in a single row on each lateral margin. Posterior labium with a single row of 16–21 marginal papillae of similar size and shape to those on anterior labium. Submarginal papillae absent. Upper jaw sheath arc-shaped, longer than lower jaw sheath, with no medial notch. Lower jaw sheath V-shaped, slightly wider than upper jaw sheath. Edge of upper and lower jaw sheaths serrated; serrations extend along the entire length of each sheath. Labial keratodont row formula (LKRF) 2(2)/3(1). Tooth row A-1 complete, 1.1 ± 0.2 mm long; A-2 is 1.1 ± 0.1 mm long and interrupted by a medial gap of 0.5 ± 0.0 mm. Tooth row P-1 is 1.0 ± 0.1 mm long with a small medial gap. Tooth row P-2 is complete and 0.9 ± 0.1 mm long. Tooth row P-3 is complete but shorter than P-1 and P-2, 0.5 ± 0.1 mm long. In preservative, dorsal, lateral and anteroventral surfaces of body cream, covered with aggregations of brown melanophores and blotches (Fig. 8). Tail fins transparent cream with brown blotches. Higher concentration of reticular-shaped blotches over tail muscle. Ventral region translucent, with some melanophores at the peripheral region and internal organs visible through the skin.

Comparisons with tadpoles of other species. Tadpoles of *Allobates velocicantus* sp. nov. have short, rounded and pyramidal papillae on each side of the labium. There are 3 or 4 papillae on the anterior labium and from 16 to 21 on the posterior labium. Hence, this species is distinct from congeners that have more-elongate papillae on the posterior labium—*A. caeruleodactylus* (6 long papillae), *A. grillisimilis* (approximately 8 very long papillae), *A. subfolionidificans* (40 papillae on the posterior labium, which are longer than those on the anterior labium) and *A. tapajos* (8–10 long papillae)—and from those that have a larger number of short papillae on the anterior labium: *A. brunneus* (5 papillae), *A. carajas* (5–8 papillae), *A. magnussoni* (12–13 papillae) and *A. nunciatus* (8–9 papillae). Tadpoles of *Allobates velocicantus* sp. nov. have an arrangement of posterior tooth rows with $P1 > P2 > P3$ and $P3$ equivalent to around half of $P2$, thus being distinct from tadpoles of *A. palevarzensis* ($P2 > P1 > P3$) and *A. hodli* ($P2 = P1 = P3$). The LKRF in tadpoles of *A. velocicantus* sp. nov. is 2(2)/3(1), which differs from the LKRF in *A. femoralis* (2(2)/3).

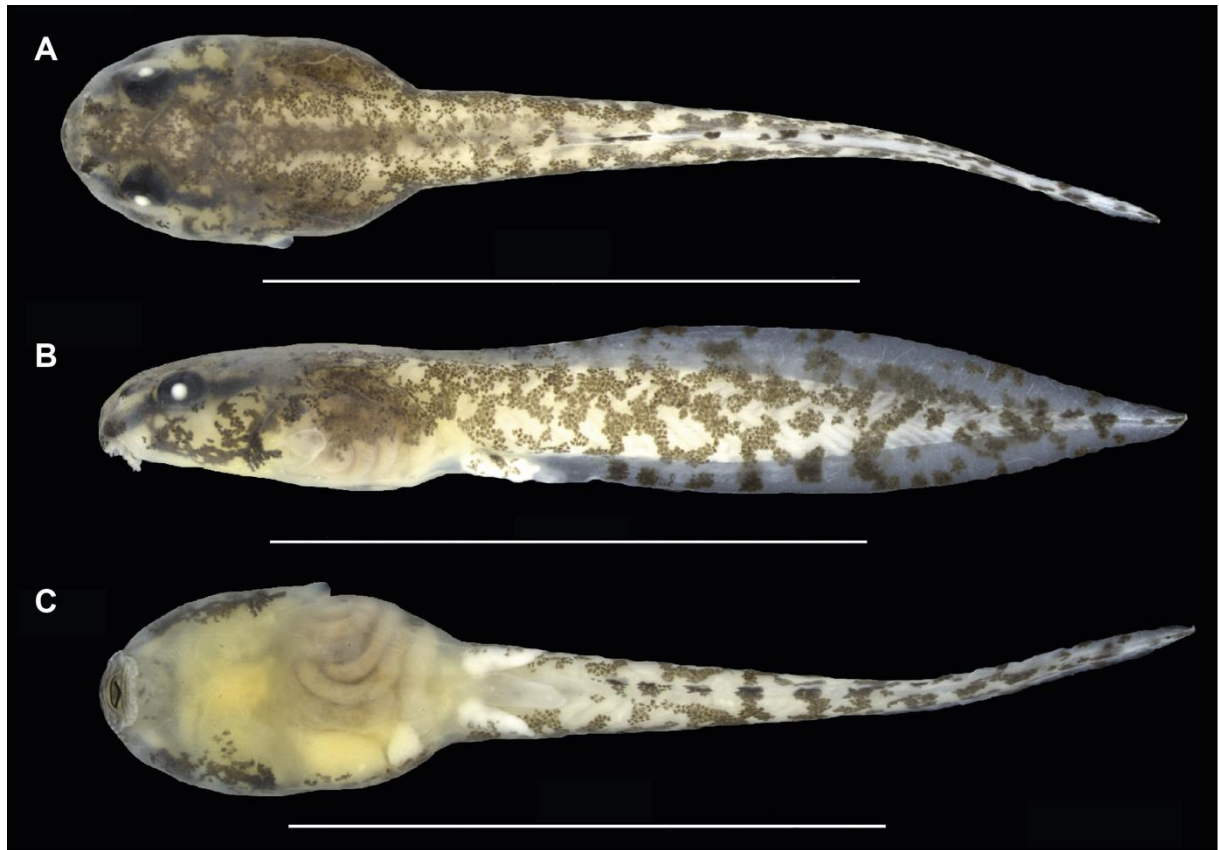


Figure 8 **Preserved tadpole of *Allobates velocicantus* sp. nov. (INPAH 41351), at Gosner stage 37.** (A) Dorsal, (B) lateral and (C) ventral views. Scale bars: 10 mm. Photographs by Jeni Lima Magnusson.

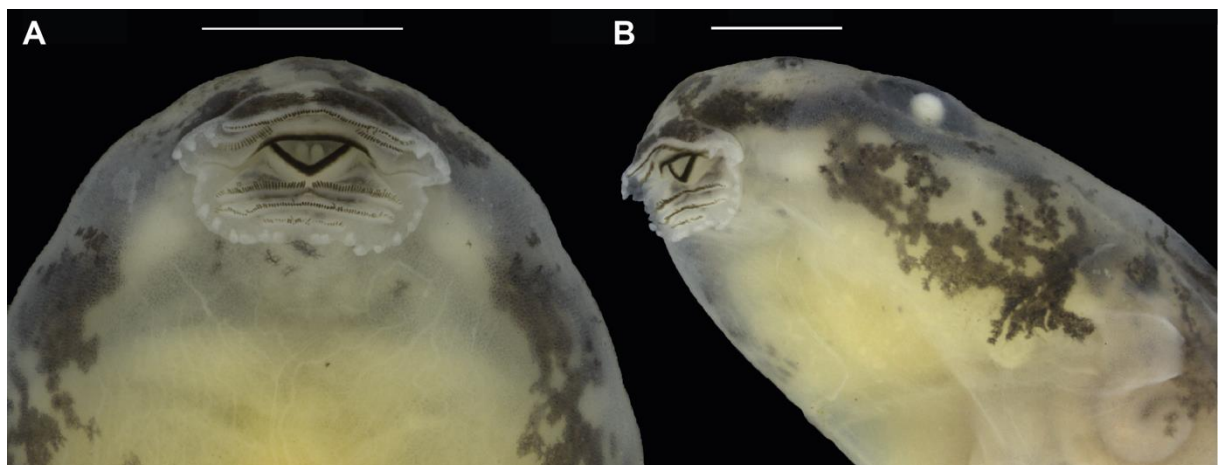


Figure 9 **Oral disc of a preserved tadpole of *Allobates velocicantus* sp. nov. (INPAH 41351).** (A) Ventral and (B) ventrolateral views. Scale bars: 1 mm. Photographs by Jeni Lima Magnusson.

Natural history notes. *Allobates velocicantus* sp. nov. inhabits the litterfall of primary and secondary lowland ombrophilous open forest (Fig. 10A). Populations of this species were found

in terra firme forest either close to or distant from small forest streams. The new species uses leaves of small shrubs in the forest understory as egg deposition sites; two clutches (nine tadpoles and 13 eggs) were found in this situation. On 13–15 February 2019, during the middle of the rainy season, males were found calling between 8:00 and 18:00 h, and always perched between 10 and 30 cm above the ground. A pair of adults was observed courting: the male was leading the female to an egg deposition site, and he emitted a courtship call while both frogs were moving. As the male started emitting another advertisement call, the female stopped following. Arriving at the egg deposition site, the male jumped on to a leaf (adaxial surface), which was located around 20 cm above the ground, and continued to emit advertisement calls interspersed with courtship calls. After a few moments, the female jumped to the leaf with the male and approached him, initially touching snouts before turning her back to him. The male then jumped onto the female's dorsum, while also sliding its hand to her head. The male remained in that position—cephalic amplexus—for 2 min before jumping from the leaf. The female remained in place for a few moments but then began moving, turning clockwise 30°, stopping, and repeating that pattern several times, always turning clockwise. The length of time the female stopped between movements ranged from 2 to 10 min, while she deposited eggs (Fig. 10C). Overall, egg deposition lasted 40 min before the female abandoned the leaf, leaving behind a clutch of 13 eggs. Each egg had a distinctly pigmented animal pole and was encased within cloudy jelly (Fig. 10D). The clutch was collected immediately after the female abandoned it, the embryos maintained alive through hatching, and the resulting tadpoles reared to Gosner stages 27–37 before being sacrificed and preserved. The second egg clutch had nine tadpoles at aquatic-transport stage. The male (INPAH 41347) jumped onto the egg clutch and made circular movements inside it, at which time the tadpoles began to wriggle towards his dorsum, eventually climbing onto it (Figs. 10E–10G). This event lasted for 5 min.

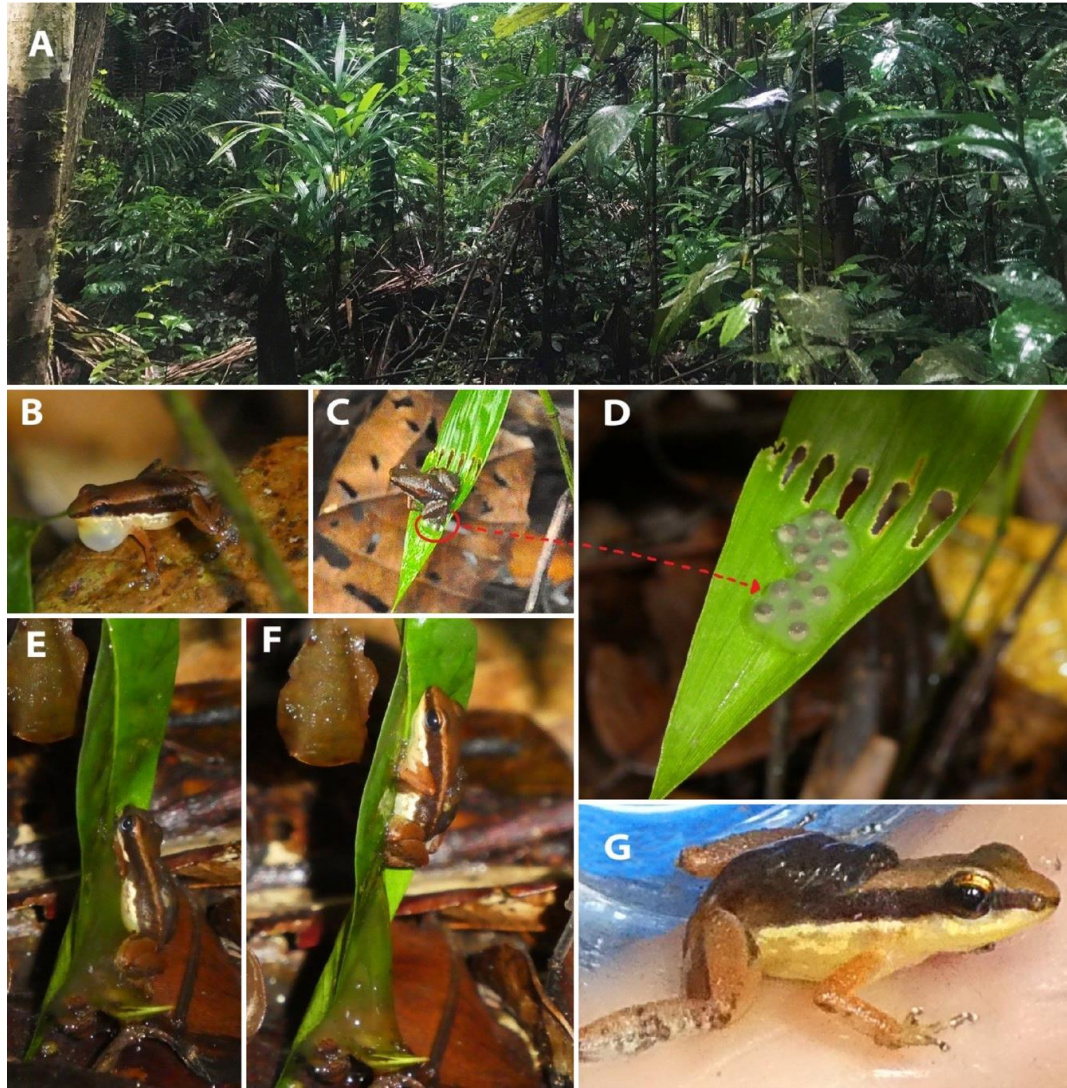


Figure 10 **Habitat and breeding behavior of *Allobates velocicantus* sp. nov.** (A) Understory of the lowland ombrophilous open forest at the type locality, municipality of Mâncio Lima, state of Acre, Brazil. (B) A calling male (INPAH 41343). (C) A female ovipositing (uncollected). (D) Egg clutch containing 13 eggs with animal pole covered by melanin. (E and F) Male collecting tadpoles to transport to a pond (INPAH 41347). (G) Male with tadpoles on his back (INPAH 41347). In both clutches, eggs were deposited on the adaxial surfaces of green leaves. Photographs by Jesus R. D. Souza.

DISCUSSION

Allobates velocicantus is the twenty-sixth species of *Allobates* described from Brazil and the twenty-third species from Brazilian Amazonia. The species is distributed in southwest Amazonia, a region of high species richness for several taxonomic groups (Jenkins, Pimm & Joppa, 2013), including anurans (Souza, 2009; Bernarde, Machado & Turci, 2011). The type locality is located in the Area of Relevant Ecological Interest Japiim Pentecostes, a conservation unit intended for sustainable use. Yet, the conservation of *A. velocicantus*, and of regional

biodiversity in general, is not guaranteed. Deforestation in this part of Amazonia, including in conservation units, has increased with the expansion of illegal logging, cattle ranching, and agriculture (Kröger, 2020; Montibeller et al., 2020). Moreover, deforestation and degradation rates in Amazonia are increasing substantially due to the anti-environmental agenda of the current Brazilian government (Ferrante & Fearnside, 2019; Kröger, 2020).

The high note-repetition rate call of *Allobates velocicantus* is a remarkable feature. Four species of *Allobates* emit calls with similar structure: *A. bacurau*, *A. crombiei*, *A. juami* and *A. insperatus*. However, the note-repetition rate in *A. velocicantus* is strikingly higher than the rates of these other species. Furthermore, the advertisement call of *A. velocicantus* has the highest number of notes among all species of *Allobates* with documented calls. These characters make the species unique and easily distinguished from congeners. While most species of *Allobates* share a conserved external morphology, vocalizations are highly variable and species-specific. They represent essential characters for use in the formal diagnosis and description of new taxa, as well as in future species discovery.

Information on egg coloration in *Allobates* is scarce except for species recently described or redescribed (Lima, Sanchez & Souza, 2007; Lima, Caldwell & Strüssmann, 2009; Lima et al., 2010; Lima, Simões & Kaefer, 2014, 2015; Moraes, Pavan & Lima, 2019; Simões et al., 2013; Simões, Rojas & Lima, 2019). *Allobates velocicantus* oviposits on green leaves of small shrubs. Currently, only two other species in Brazilian Amazonia use this breeding strategy exclusively: *A. subfolionidificans* and *A. carajas*. Unlike in *A. velocicantus*, however, the animal pole of eggs in these two species is white (Lima, Sanchez & Souza, 2007; Souza, Kaefer & Lima, 2017; Simões, Rojas & Lima, 2019). Despite both *A. velocicantus* and *A. subfolionidificans* inhabiting the open-canopy lowland forest with considerable incidence of sun rays above canopy, eggs of *A. subfolionidificans* are protected from direct solar radiation by being deposited on the abaxial surfaces of green or dry leaves of small shrubs (Lima, Sanchez & Souza, 2007; Souza, Kaefer & Lima, 2017), while *A. velocicantus* oviposits on the adaxial surface of green leaves of small shrubs. On the other hand, *A. carajas* oviposits white eggs on the adaxial surface of green leaves (Simões, Rojas & Lima, 2019), but eggs are protected by a dense-canopy forests with low levels of solar incidence on the lower understory vegetation (P.I. Simões, 2014, personal communication). Intense solar radiation may cause mortality and abnormal embryo development in anurans (Beudt, 1930; Gurdon, 1960; Blaustein et al., 1997), so the amount of melanin on eggs in these species might be an ecophysiological response to the solar radiation to which eggs are exposed. Differences in the amount of melanin on eggs were

reported from other two species of the *A. tinae* species complex inhabiting forests with distinct canopy openness in the Brazilian Amazonia (Lima, Ferrão & Silva, in press). The color of jelly may have a protective function against solar radiation. Jelly of *A. velocicantus* is cloudy, while the jelly of *A. subfolionidificans* and *A. carajas* are translucent. The effect of solar radiation on eggs of *Allobates* with different concentrations of melanin and deposited within jellies of different colors needs physiological tests.

CONCLUSION

Allobates velocicantus is differentiated from its congeners based on external morphology of adults and tadpoles, advertisement call and molecular analyses. The species represents an excellent model to study the ecological and physiological adaptations to solar radiation on eggs of *Allobates*. However, the conservation of *A. velocicantus* is threatened by the expansion of illegal logging, cattle ranching, and agriculture.

APPENDIX I. SPECIMENS EXAMINED

***Allobates tinae*.** Adults. Brazil: Rondônia: Porto Velho, west bank of upper Madeira River [INPAH 41012–21, 41029–36, 41041–44]; Amazonas: Boca do Acre (INPAH 40976, 41022, 41027, 41037, 41040).

***Allobates aff. tinae*.** Adults. Brazil: Amazonas: Careiro, RAPELD M1 at km 32 of the Brazilian federal highway BR-319 [INPAH 41045–47, 41049–53, 41055–57, 41059, 41061, 41063, 41067–69].

***Allobates bacurau*.** Adults. Brazil: Amazonas: Estrada do Miriti, Manicoré [INPAH 35398 (holotype); 35397, 35399–35409 (paratypes)].

***Allobates brunneus*.** Adults. Brazil: Mato Grosso: NE of Chapada dos Guimarães [INPAH 10111–48 (topotypes)]. Tadpoles. Brazil: Mato Grosso: NE of Chapada dos Guimarães. INPAH 10025–10027, 10029–10030, 10032–10037, 10039, 10041, 10043, 10044 (topotypes).

***Allobates caeruleodactylus*.** Adults. Brazil: Amazonas: km 12 on the road to Autazes [INPAH 7238 (holotype); 7229–7232, 7234–7237 (paratypes)]. Tadpoles. Brazil: Amazonas: km 12 on the road to Autazes (INPAH 8037–8046, 8085).

***Allobates crombiei*.** Adults. Brazil: Pará: Cachoeira do Espelho [INPAH 30457–30477 (topotypes)].

***Allobates femoralis*.** Adults. Brazil: Pará: Treviso (INPAH 11657–11671, 15232,

30769–30778); Pará: Itaituba (INPAH 26342–26354).

Allobates fuscus. Adults. Brazil: Amazonas: Ipixuna: Penedo, east bank of Juruá river [INPAH 2531 (paratopotype); 2532 (holotype)]; Itamarati: Jainu, Juruá River [INPAH 3114, 3250, 3270, 3514 (paratypes)].

Allobates gasconi. Adults. Brazil: Amazonas: Itamarati: Jainu, west bank of Juruá River [INPAH 3082 (holotype); 3073, 3079, 3085, 3090, 3150, 3151, 3172, 3249, 3406, 3415, 3483, 3484, 3491, 3494, 3496, 3512, 3513 (paratypes)].

Allobates grillisimilis. Adults. Brazil: Amazonas: Borba [INPAH 30779 (holotype); 30780–30808 (paratopotypes)]; Nova Olinda do Norte [INPAH 30809–30823 (paratypes)].

Allobates magnussoni. Adults. Brazil: Pará: Parque Nacional da Amazônia [INPAH 32960 (holotype); 32961–32976, 32978–32982 (paratopotypes)]; Treviso (INPAH 10105–10109, 33930–33934). Tadpoles. Brazil: Pará: Treviso (INPAH 10054, 10056, 10058, 10059, 10060).

Allobates masniger. Adults. Brazil: Amazonas: Borba (INPAH 28075, 28078, 28084, 28089, 28092, 28095, 28098, 28100, 28104, 28105, 28112, 28114, 28119); Pará: Parque Nacional da Amazônia [INPAH 28195–28217 (topotypes)]; Jacareacanga (INPAH 28053, 28070, 28077, 28082, 28093, 28094, 28099, 28103, 28107, 28110, 28111, 28113, 28115, 28118, 28120).

Allobates nidicola. Adults. Brazil: Amazonas: km 12 on road to Autazes [INPAH 8093 (holotype); INPAH 7253–7259, 7261, 7262, 8094 (paratypes); INPAH 28122, 28124, 28127, 28129, 28131, 28144, 28159, 28163, 28166, 28169, 28171, 28172, 28174, 28179, 28184, 28185 (topotypes)]. Tadpoles. (INPAH 8021–8033, 8137–8139).

Allobates nunciatus. Adults. Brazil: Pará: Itaituba [INPAH 40486 (holotype); INPAH 40305, 40307, 40475, 40480, 40485, 40489, 40324, 40476 (paratypes)]; Trairão [INPAH 40482, 40484, 40488 (paratypes)].

Allobates paleovarzensis. Adults. Brazil: Amazonas: Careiro da Várzea [INPAH 20904 (holotype); INPAH 20861–20903, 20905 (paratypes)].

Allobates subfolionidificans. Adults. Brazil: Acre: Parque Zoobotânico da Universidade Federal do Acre [INPAH 13760 (holotype); INPAH 11958–11974, 13749–13754, 13756–13759, 13761, 13762 (paratypes)]. Tadpoles. Brazil: Acre: Parque Zoobotânico da Universidade Federal do Acre (INPAH 14822, 14823).

Allobates tapajos. Adults. Brazil: Pará: Parque Nacional da Amazônia [INPAH

34402–34424 (paratypes); INPAH 34425 (holotype)]. Tadpoles. Brazil: Pará: Parque Nacional da Amazônia (Lots INPAH 34426, 34427).

Allobates trilineatus. Adults. Brazil: Acre: Parque Zoobotânico da Universidade Federal Do do Acre (INPAH 11958–11993).

Allobates vanzolinius. Adults. Brazil: Amazonas: Vai-Quem-Quer, Rio Juruá [INPAH 4896 (holotype); INPAH 4903, 4904, 4905, 4912 (paratypes)]; Jaiú, Rio Juruá [INPAH 3381, 3413 (paratypes)].

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Jesus R.D. Souza conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Miquéias Ferrão performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- James Hanken analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Albertina P. Lima conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers): Specimens were collected under collection permit number 1337-1 provided by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). Protocols of collection and animal care follow the Conselho Federal de Biologia resolution number 148/2012 (CFB, 2012).

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers): Specimens were collected under collection permit number 1337-1 provided by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA).

Data Availability

The following information was supplied regarding data availability: Specimens are housed in the herpetological section of the Zoological Collection of the Instituto Nacional de Pesquisas da Amazônia (INPAH), Manaus, Amazonas, Brazil, under numbers INPAH 41339 to INPAH 41351. Sequences are available at GenBank under accession numbers MT446458 to MT446462. Morphometric measurements are provided in a Supplemental Table. Advertisement calls are housed at Fonoteca Neotropical Jacques Vielliard (FNJV 45469–79), UNICAMP, Campinas, Brazil. In addition, call recordings are available at Figshare: Souza, Jesus; Ferrão, Miquéias; Hanken, James; Lima, Albertina Pimentel (2020): Advertisement call of a new species of *Allobates* from Acre, Brazil. figshare. Media. DOI 10.6084/m9.figshare.12283973.v1.

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Supplemental Information

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Allobates albiventris sp. nov.



CAPÍTULO 2

Jesus R.D. Souza, Miquéias Ferrão, Igor Luis Kaefer, Antonio Saulo Cunha-Machado, Paulo Roberto Melo-Sampaio, James Hanken e Albertina P. Lima. A new pale-ventered nurse frog (Aromobatidae, *Allobates*) from southwestern Brazilian Amazônia. Manuscrito aceito para publicação no periódico Vertebrate Zoology em 05 de junho de 2023. ID 103534.

A new pale-ventered nurse frog (Aromobatidae, *Allobates*) from southwestern Brazilian Amazonia

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Abstract

We use integrative taxonomy to formally describe a candidate species of nurse frog of the genus *Allobates* from southwestern Brazilian Amazonia. The new species nest within a clade that has been defined historically as *A. gasconi*, but it has an 8.8–11.0% genetic distance for 16S to samples from the type locality of *A. gasconi*. The new species differs from congeners mainly by males having a translucent white throat and vocal sac; advertisement calls with a duration of 42–60 ms, two notes separated by an inter-note interval of 8–23 ms, and a dominant frequency of 4,953–6,331 Hz; and exotrophic tadpoles with 2 pyramidal papillae on each end of the upper lip and 10–13 pyramidal and cylindrical papillae surrounding the lower lip. Phylogenetic analyses based on mitochondrial DNA suggests that *A. gasconi* sensu lato as defined previously represents a complex of as many as seven species, corroborating studies that have shown high levels of cryptic diversity within *Allobates*.

Key words: advertisement call, biodiversity, integrative taxonomy, morphology, phylogeny, reproductive behavior, state of Acre, tadpole

Introduction

Nurse frogs of the genus *Allobates* Zimmermann and Zimmermann, 1988 are leaf-litter inhabitants of neotropical forests distributed from Central America to the east coast of South America (Grant et al. 2006, 2017; Frost 2023). Diverse reproductive behavior among species is associated with several different reproductive modes (*sensu* Nunes-de-Almeida et al. 2021), with most species depositing eggs on leaf-litter (e.g., *A. caldwella* and *A. tapajos*; Lima et al. 2015, 2020) but others depositing them on the adaxial (e.g., *A. carajas* and *A. velocicantus*; Simões et al. 2019; Souza et al. 2020) or abaxial (e.g., *A. subfolionidificans*; Lima et al. 2007) surface of live leaves of small shrubs. Clutch size varies among species, as does coloration of eggs and the surrounding jelly (Lima et al. 2020). Parental care is common to all *Allobates*; species with exotrophic, free-swimming tadpoles exhibit clutch care and tadpole transport (e.g., *A. femoralis*), while species with endotrophic terrestrial tadpoles exhibit only clutch care (e.g., *A. nidicola*, *A. masniger*; Caldwell and Lima 2003).

Advertisement calls may be classified into two main types following the note-centered approach (*sensu* Köhler et al. 2017): those with two or more notes (e.g., *A. flaviventris* and *A. trilineatus*; Melo-Sampaio et al. 2013; Jaramillo et al. 2021) and those with a single note (e.g., *A. nidicola* and *A. paleovarzensis*; Caldwell and Lima 2003; Lima et al. 2010). Most species

are cryptically colored and exhibit little morphological divergence, which contrasts sharply with extensive genetic, behavioral and bioacoustic divergence that characterizes most interspecific comparisons (Kaefer et al. 2013; Maia et al. 2017; Fernandes et al. 2021).

The recent increase in the understanding of taxonomic diversity of *Allobates* is impressive: at least thirteen species have been described in just the last five years (Melo-Sampaio et al. 2018, 2020; Simões et al. 2018, 2019; Moraes et al. 2019; Lima et al. 2020; Souza et al. 2020; Gagliardi-Urrutia et al. 2021; Moraes and Lima 2021; Silva et al. 2022; Ferrão et al. 2022; Fouquet et al. 2023). This trend may be attributed to the increased sampling of remote areas of Amazonia and to the use of integrative taxonomy (e.g., Simões et al. 2018, 2019; Lima et al. 2020; Souza et al. 2020; Silva et al. 2022). These advances in alpha taxonomy and sampling coverage have led to new biogeographic hypotheses about the origin and diversification of this diverse genus (e.g., Réjaud et al. 2020). Nevertheless, many studies have concluded that species diversity in *Allobates* remains underestimated and that, in particular, some widely distributed species (e.g., *A. gasconi*, *A. granti*, *A. tapajos*, *A. tinae* and *A. trilineatus*) likely represent complexes of multiple species, each with a more restricted geographic distribution (Lima et al. 2020; Vacher et al. 2020; Jaramillo et al. 2021; Ferrão et al. 2022; Fouquet et al. 2023).

Allobates gasconi was described based on the morphology of 33 individuals from five localities along both banks of the Juruá River, southwestern Brazilian Amazonia (Morales 2002). Several subsequent studies published genetic sequences tentatively identified as *A. gasconi*. Grant et al. (2006) published the first phylogenetic inference that included *A. gasconi*, based on individuals collected from the Ituxi River, an eastern tributary of the upper Purus River and ~400 km from the type locality in the Juruá River. Santos et al. (2009) subsequently applied the name *A. gasconi* to individuals from Porto Walter in the upper Juruá River (Acre, Brazil), which were recently assigned to *A. trilineatus* (Melo-Sampaio et al. 2018). Lima et al. (2014) sequenced individuals from the middle Juruá River (Eirunepé, Amazonas, Brazil) and identified them as *A. gasconi*. Melo-Sampaio et al. (2018) compiled these molecular data, corrected misidentifications and sequenced new samples from several localities in the upper Juruá and Purus basins (Acre and Amazonas, Brazil), recovering three deeply diverging mtDNA lineages within *A. gasconi* sensu lato. More recently, Vacher et al. (2020) sequenced new individuals of *A. gasconi* from the upper Madeira River (Rondônia, Brazil) and showed the existence of at least three highly distinct mtDNA lineages. However, their analysis did not include the newly sequenced individuals reported by Melo-Sampaio et al. (2018) from Acre. Réjaud et al. (2020)

delimited all these lineages as a single species and recovered it as a member of the *A. caeruleodactylus* clade. Recently, Ferreira et al. (in press) redescribed *A. gasconi* based on fresh material from type and paratype localities and showed that none of those individuals identified as *A. gasconi* in earlier studies indeed represent *A. gasconi* sensu stricto. Moreover, Ferreira et al. (in press) highlighted that *A. gasconi* sensu lato comprises at least three candidate species and that none is closely related phylogenetically to *A. gasconi* sensu stricto.

We recently surveyed multiple localities in southwestern Amazonia (Acre, Brazil). Among the material collected are several individuals of *Allobates gasconi* sensu lato that nest within a previously reported lineage by Melo-Sampaio et al. (2018) and Ferreira et al. (in press). In this study, we describe them as a new species based on morphological, bioacoustic, and molecular data, as well as their breeding behavior.

Materials and methods

Sampling

Adult individuals were collected between November 2018 and February 2020 from five localities in southwestern Brazilian Amazonia—four in the state of Acre (forest fragment near the BR 364 highway, municipality of Manoel Urbano [8°52'27" S, 69°17'07" W, 166 meters above sea level—hereafter masl]; east bank of the Antimary River, municipality of Bujari [09°29'16" S, 68°21'20" W, 168 masl]; Parque Ambiental Chico Mendes, municipality of Rio Branco [10°02'13" S, 67°47'36" W, 158 masl]; and municipality of Feijó [08°14'13" S, 70°22'44" W, 184 masl]) and a fifth in the state of Amazonas (Reserva Extrativista Arapixi, municipality of Boca do Acre [08°58'21" S, 67°51'50" W, 125 masl]). Ten tadpoles (field number APL21518b) were taken from the dorsum of a male (APL21518) collected in Manoel Urbano on January 18, 2020; 18 eggs were taken from a freshly laid spawn (field number APL21511b) on January 7, 2020.

Tadpoles and eggs were reared in the laboratory until they reached Gosner (1960) developmental stages 33–37. Adults were killed with 2% aqueous benzocaine, fixed in 10% neutral-buffered formalin (NBF), and preserved in 70% ethanol. Tadpoles were killed with 2% aqueous benzocaine diluted in water and fixed in 10% NBF. Tissue samples for genetic analysis were removed before fixation and stored in 95% ethanol. Adults and tadpoles are deposited in herpetological collections at the Harvard Museum of Comparative Zoology (**MCZ**; Cambridge, USA), Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (**MCP**; Porto Alegre, Brazil), Instituto Nacional de Pesquisas da Amazônia (**INPA-H**;

Manaus, Brazil), Museu Nacional (**MNRJ**; Rio de Janeiro, Brazil) and Museu Paraense Emílio Goeldi (**MPEG**; Belém, Brazil).

Advertisement calls of 17 males were recorded: eight from Manoel Urbano, two from Antimary River, three from Parque Ambiental Chico Mendes, one from Feijó and three from Reserva Extrativista Arapixi. Recordings were made between 0630 and 1100 h and between 1500 and 1730 h using a Sony PCM-D50 digital recorder with built-in microphone, a CSR Yoga HT-81 Shotgun directional microphone attached to a Zoom H4n recorder, and a Sennheiser ME 66 unidirectional microphone attached to a Marantz PMD661 digital recorder. Microphones were positioned approximately 1 m from the focal male. Recordings were taken at 16-bit resolution and 44.1 kHz and stored in WAV format. Air temperature recorded at the time of recordings ranged from 21 to 29°C. Recordings are deposited in the Fonoteca Neotropical Jacques Vielliard (**FNJV** 53680–93), UNICAMP, Campinas, Brazil.

Sequencing and phylogenetic analysis

Total genomic DNA was extracted from tissue samples of five individuals of the new species from Manoel Urbano, two from Antimary River and two from Parque Ambiental Chico Mendes (Rio Branco). DNA extractions were obtained using the commercial kit Wizard (Promega Corp., Madison, WI, USA) following manufacturer's instructions. Fragments of the 16S rRNA mitochondrial gene were amplified by polymerase chain reaction (PCR) using the universal primers 16sar (CGCCTGTTTATCAAAAACAT) and 16sbr (CCGGTCTGAACTCAGATCACGT; Palumbi 1996). The PCR with final volume of 15 µL contained 6.4 µL distilled and deionized water; 1.5 µL MgCl (25 mM); 1.5 µL Tris-HCl buffer (10 mM); 1.5 µL dNTPs (25 mM); 1.5 µL 16sar primer at 2 pmol/µL; 1.5 µL 16sbr primer at 2 pmol/µL; 0.1 µL taq DNA polymerase (5 U/µL); and 1 uL DNA (10 ng/µL). The following thermocycling process was used for amplifying the reaction: 92°C for 30 s for the initial denaturation, 35 cycles of denaturation at a temperature of 92°C for 10 s, annealing at 50°C for 35 s, and extension at 72°C for 90 s; the final extension was performed at 72°C for 10 min. PCR products were purified with PEG (polyethylene glycol) 8000 and sequenced using the Big Dye Terminator Kit (Applied Biosystems, Waltham, USA) in an automated sequencer ABI Prism 3130 (ThermoFisher Scientific, Waltham, USA). Sequences were visually checked and manually edited in GENEIOUS 5.3.4 (Kearse et al. 2012). Newly generated sequences are deposited in GenBank (www.ncbi.nlm.nih.gov/Genbank) under accession numbers ON937745–53.

Sequences of the 16S fragment and four additional genes (12S ribosomal RNA [12S], cytochrome c oxidase I [COI], cytochrome b [CYTB] and NADH dehydrogenase 1 [ND1]) were retrieved from GenBank to infer the phylogenetic relationship of the new species, including sequences of five individuals of the new species previously reported as *A. gasconi* by Melo-Sampaio et al. (2018). Sequences of *Ameerega* Bauer, 1986, *Anomaloglossus* Grant et al. 2006, *Aromobates* Myers et al. 1991, *Colostethus* Cope, 1866, *Dendrobates* Wagler, 1830, *Leucostethus* Grant et al. 2017, *Mannophryne* La Marca, 1992, *Phyllobates* Bibron, 1840, *Rheobates* Grant et al. 2006 and *Silverstoneia* Grant et al. 2006 were used as outgroups. The final dataset combined 104 sequences of 16S, 60 of 12S, 50 of COI, 64 of CYTB and 41 of ND1. Sequences were aligned separately for each gene with the online implementation of the MAFFT algorithm (<https://mafft.cbrc.jp/>) using the E-INS-i strategy for RNA genes and G-INS-i for the protein-coding genes (Katoh and Standley, 2013). MESQUITE 3.04 (Maddison and Maddison 2015) was used to concatenate alignments and generate the final matrix, which is composed of 6,251 base-pairs and 104 terminals. Appendix 1 lists vouchers and GenBank accession numbers for the entire dataset.

The final matrix was split into 10 partitions, one for RNA genes (12S plus 16S) and one for each codon of protein-coding genes (COI, CYTB and ND1). The following best-fit partitioning scheme and nucleotide models were inferred with MODELFINDER (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE (Nguyen et al. 2015): GTR+F+R6 for 12S+16S, SYM+I+G4 for CYTB 1st codon plus ND1 1st codon, TIM+F+I+G4 for CYTB 2nd codon plus ND1 2nd codon plus COI 2nd codon, TN+F+R5 for CYTB 3rd codon plus ND1 3rd codon plus COI 3rd codon, and TIM3e+I+G4 for COI 1st codon. Phylogenetic relationship was inferred through Maximum Likelihood inference using IQ-TREE. Node support was calculated using 10,000 ultrafast bootstrap approximation replicates (Hoang et al. 2018) with a maximum 10,000 iterations (unsuccessful iterations to stop > 100), a 0.99 minimum correlation coefficient and 10,000 replicates of the Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT). Uncorrected (p) and Kimura-2-parameter (K2p) distances (Kimura 1980) between the new species and other nurse frogs were calculated with MEGA 11 (Tamura et al. 2021).

Molecular species delimitation

We conducted three molecular species delimitation analyses to delimit putative species: Generalized Mixed Yule Coalescent (GMYC; Pons et al. 2006; Fujisawa and Barraclough 2013), Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021) and

Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012). GMYC delimits putative species by optimizing the set of nodes that define the transitions between inter- and intra-specific processes through speciation (Yule 1925) and neutral coalescent (Hudson 1990) models (Pons et al. 2006; Fujisawa and Barraclough 2013). ASAP and ABGD are clustering algorithms that implement pairwise genetic distances (Jukes-Cantor, K2P or p distances) to estimate multiple potential barcode gaps and define minimum distance thresholds to partition samples into putative species. The main difference between them is that the best partition is selected through a score system in ASAP, while in ABGD this selection is unsupervised and based on prior knowledge regarding interspecific genetic distance thresholds. As delimitations may differ between methods, a lineage was considered a candidate species if it was supported by at least two methods (majority rule consensus).

Molecular species delimitation analyses were conducted with the complete 16S alignment, which include all other species of *Allobates* included in previous phylogenetic analyses. GMYC requires a single-locus ultrametric phylogenetic tree as input, therefore a 16S tree was inferred through Bayesian Inference in BEAST 2.6.3 (Bouckaert et al. 2019) using a relaxed log-normal clock model and Yule Process tree prior; a run of 100 million generations was sampled every 10,000 steps. Stationarity and effective sample size (> 200) of posterior distributions and priors were checked in TRACER v1.7.1 (Rambaut et al. 2018). The Maximum Clade Credibility (MCC) tree was annotated using TREEANOTATOR v.2.6.3 (Bouckaert et al. 2019) after burn-in of 40%. The GMYC was run in the webserver <https://species.h-its.org/gmyc/> using the single threshold. ASAP was run in the webserver <https://bioinfo.mnhn.fr/abi/public/asap/> using p distances as the substitution model and default parameters. ABGD was run in the webserver <https://bioinfo.mnhn.fr/abi/public/abgd> using p distances as the sequence substitution model, minimum intraspecific pairwise distance = 0.001 and maximum = 0.1, 10 iterative steps, and relative gap width (X) = 1. ASAP and ABGD were run with the 16S alignment.

Morphological characters

Sex was determined by the presence of a vocal sac or direct observation of vocal activity in males, and by the presence of eggs (visible through belly skin) in females. The following linear measurements, taken using a digital calipers (precision 0.01 mm) or a stereomicroscope (precision greater than 0.1 mm), follow Lima et al. (2007): snout-vent length (**SVL**); head length (**HL**); interorbital distance (**IO**); head width (**HW**); snout length (**SL**); eye-nostril

distance (**END**); inter-nostril distance (**IND**); eye length (**EL**); tympanic diameter (**TYM**); forearm length (**FAL**); upper arm length (**UAL**); thigh length (**LL**); tibia length (**TL**); foot length (**FL**); hand length from the proximal edge of the palmar tubercle to the tip of finger I (**HANDI**), finger II (**HANDII**), finger III (**HANDIII**) and finger IV (**HANDIV**); width of finger III disc (**WFD**); palmar tubercle diameter (**DPT**); width of thenar tubercle (**WTT**); width of finger III at proximal phalanx (**WPF**); and width of toe IV disc (**WTD**). Terminology and morphological characters follow Grant et al. (2017), except for finger numbering (I–IV). Character states of swelling on finger III follows Cavalcanti et al. (2022). Morphological descriptions follow Souza et al. (2020). Coloration is described from photographs and fieldnotes.

Developmental stage was scored according to Gosner (1960). Nine tadpoles were measured from lot APL21518b and three from lot APL21511b, all at stages 34–37. The following linear measurements, taken with a micrometer coupled to a stereomicroscope, follow Altig and McDiarmid (1999) and Schulze et al. (2015): total length (**TL**); body length (**BL**); tail length (**TAL**); body width (**BW**); body height (**BH**); head width (**HWLE**); tail muscle width (**TMW**); maximum tail height (**MTH**); tail muscle height (**TMH**); interorbital distance (**IOD**); inter-nostril distance (**IND**); eye-nostril distance (**END**); nostril-snout distance (**NSD**); eye diameter (**ED**); vent-tube length (**VTL**); spiracle-tube length (**STL**); and oral-disc width (**ODW**). Terminology and diagnostic characters follow Altig and McDiarmid (1999) and Schulze et al. (2015). Characterization of body shape follows Dubeux et al. (2020). Morphological descriptions are based on six tadpoles at stage 34 and follow Schulze et al. (2015). Coloration is described from photos of formalin-fixed specimens. Morphometric measurements are listed in Supplementary Table 1.

Morphological analysis

Ferreira et al. (in press) show that *Allobates gasconi* sensu stricto and the new species (as *A. gasconi* SL4) are not closely related. However, they have been misidentified historically. Due to that, we also took the above morphometric measurements from topotypic adults of *A. gasconi* recently collected by Ferreira et al. (in press) to allow direct comparisons between them and adults of the new species. The type series of *A. gasconi* is considerably dehydrated (Ferreira et al. in press). To avoid biasing the statistical analysis with potentially confounding data, we did not use type specimens.

Although body size be informative in some interspecific pair-wise comparisons, statistical artifacts are eventually associated the use of raw body ratios (all measurements against SVL) in standard Principal Component Analysis (PCA), thus we ran an adapted PCA called Shape PCA (Baur and Leuenberger 2011). Briefly, raw morphometric measurements are first log-transformed and then standardized using log-mean of each variable to avoid nonlinearity issues. Then, size and shape algorithms are implemented to allow the interpretation of principal components (PC) in terms of body shape and isometric size (isosize). Isosize is the geometric mean of all morphometric measurements of an individual (Baur and Leuenberger 2011). Shape PCA was run separately for males and females using the R script available at Zenodo (DOI: 10.5281/zenodo.3890195; Baur and Leuenberger 2020). To test if body shape and isosize of *A. gasconi* sensu stricto differ statistically from those of the new species, one-way analysis of variance (one-way ANOVA) was run separately for shape PC1, shape PC2 and isosize, setting them as response variables and species as factors. Statistical significance was calculated through Tukey's test using $\alpha = 0.05$. Morphometric measurements of both species are available at Supplementary Table S2.

Presence of sexual dimorphism in SVL and 22 morphometric ratios of the new species was evaluated with one-way ANOVAs through the function *aov* of the package *stats* (R Core Team 2021). Homogeneity of variances was checked visually with Residuals vs. Fitted values Plot and tested with Levene test using the function *leveneTest* of the *car* package (Fox and Weisberg 2019). Normality of residuals was inspected with Normal Q-Q plots and tested with the Shapiro-Wilk test through the function *shapiro.test* of the *stats* package. Outliers were excluded from analyses to fit homogeneity and normality assumptions. Multiple comparisons adjustment of p values was calculated using Benjamini and Hochberg correction (Benjamini and Hochberg 1995) through the function *p.adjust* of the package *stats*.

Bioacoustic analysis

Terminology used to describe the advertisement call of the new species follows the note-centered approach (Köhler et al. 2017). The following spectral and temporal parameters were measured from ten calls of 17 males: call duration (**CD**), inter-call interval (**ICI**), call-series duration (**CSD**), inter-call-series interval (**ICSI**), note duration measured in the first (**ND1**) and second (**ND2**) notes, inter-note interval (**INI**), lower (**LFN1**, **LFN2**), upper (**HFN1**, **HFN2**) and dominant (**DFN1**, **DFN2**) frequency of the first and second notes, respectively. All recordings were visually inspected to count the proportion of each call arrangement in the

vocalization of the new species. Acoustic analyses were performed in Raven pro 1.5 (Bioacoustics Research Program 2015) set as follows: window = Blackman, Discrete Fourier Transform = 2,048 samples and 3 dB filter bandwidth = 80.0 Hz. Dominant frequency was measured using the Peak frequency function; upper and lower frequencies were measured 20 dB below the peak frequency to avoid background noise interference. Graphical representation of calls was generated in R using the Seewave package (Sueur et al. 2008) configured as follows: Hanning window, overlap of 85% and 256 points of resolution (FFT).

Breeding behavior

Notes on reproductive behavior were obtained from observation of four courtship events that occurred between 0600 and 0900 h: one in Manoel Urbano on February 7, 2020; the other three in Parque Ambiental Chico Mendes on February 12, 14 and 15, 2020. The courting male APL21511 and female APL21512 were collected; both are included in the type series.

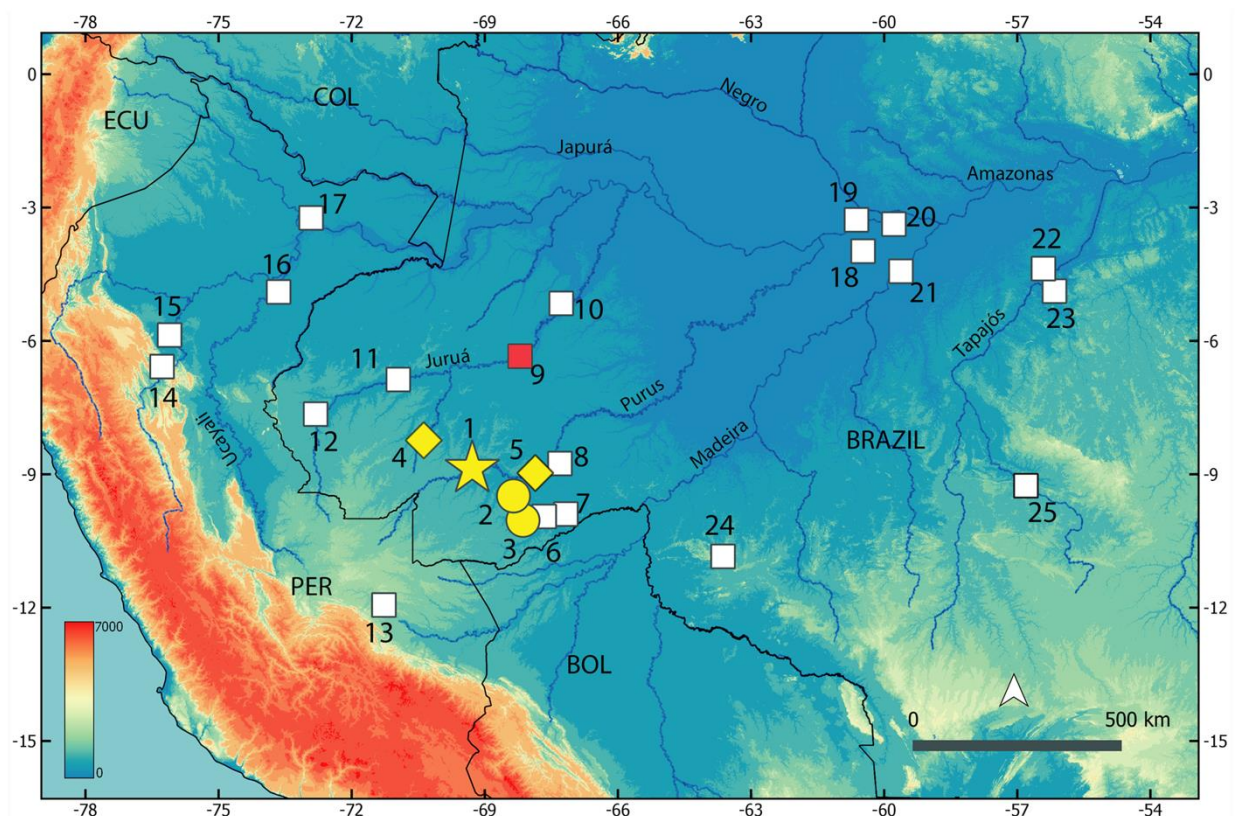


Figure 1. Geographic distribution of the new species (yellow symbols): Type locality = star (1, Manoel Urbano), paratype localities = circles (2, Antimary River; 3, Parque Ambiental Chico Mendes) and additional localities = rhombus (4, Feijó; 5, Reserva Extrativista Arapixi). Type localities of other *Allobates* to which it is compared (squares): 6, *A. subfolionidificans*; 7, *A. flaviventris*; 8, *A. tinae*; 9, *A. gasconi* (red square); 10, *A. vanzolinus*; 11, *A. fuscillus*; 12, *A. velocicantus*; 13, *A. conspicuus*; 14, *A. ornatus*; 15, *A. trilineatus*; 16, *A. sieggreenae*; 17, *A. melanolaemus*; 18, *A. caeruleodactylus*; 19, *A. nidicola*; 20, *A. paleovarzensis*; 21, *A.*

grillisimilis; 22, *A. tapajos*; 23, *A. grillicantus*; 24, *A. pacaas*; 25, *A. paleci*; 26, *A. kamilae*. Abbreviations: BOL, Bolivia; COL, Colombia; ECU, Ecuador; PER, Peru.

Interspecific comparisons

The new species has been found only in the upper Juruá and Purus basins, southwestern Brazilian Amazonia. Since most *Allobates* have restricted geographic distributions when compared to species in other genera, interspecific comparisons here are limited to cryptically colored species known to be distributed in the southwestern Amazonian lowlands of Bolivia, Brazil and Peru (Appendix 2): *A. conspicuus* (Morales, 2002); *A. flaviventris* Melo-Sampaio, Souza and Peloso, 2013; *A. fuscillus* (Morales, 2002); *A. gasconi* (Morales, 2002); *A. kamilae* Ferrão, Hanken and Lima, 2022; *A. melanolaemus* (Grant and Rodríguez, 2001); *A. nidicola* (Caldwell and Lima, 2003); *A. ornatus* (Morales, 2002); *A. pacaas* Melo-Sampaio et al. 2020; *A. paleovarzensis* Lima, Caldwell, Biavati and Montanarin, 2010; *A. sieggreenae* Gagliardi-Urrutia, Castroviejo-Fisher, Rojas-Runjaic, Jaramillo, Solís and Simões, 2021; *A. subfolionidificans* (Lima, Sanchez and Souza, 2007); *A. tinae* Melo-Sampaio, Oliveira and Prates, 2018; *A. trilineatus* (Boulenger, 1884); *A. vanzolinus* (Morales, 2002); and *A. velocicantus* Souza, Ferrão, Hanken and Lima, 2020. In addition, we make comparisons to species of the *A. caeruleodactylus* clade (*sensu* Réjaud et al. 2020): *A. caeruleodactylus* Lima and Caldwell, 2001; *A. grillisimilis* Simões, Sturaro, Peloso and Lima, 2013a; *A. grillicantus* Moraes and Lima, 2021; *A. paleci* Silva, Marques, Folly and Santana, 2022; and *A. tapajos* Lima, Simões and Kaefer, 2015. Corresponding type localities are plotted in Figure 1.

Results

Phylogenetic relationships

The topology of our molecular phylogeny is consistent with that recovered in the most recently published phylogeny of *Allobates* (Réjaud et al. 2020), particularly with respect to the relationships among major clades (Supplementary Figure 1). Three major clades compose the *A. caeruleodactylus* clade (Fig. 2): one grouping *A. caeruleodactylus*, *A. grillicantus* and *A. grillisimilis*; another one nesting members of the *A. tapajos* species complex; and the last one grouping species previously misidentified with *A. gasconi*. The last two clades are recovered as sisters with high support. The new species (*A. albiventris* **sp. nov.**) nests within the species complex that groups species previously misidentified as *Allobates gasconi*, which is called *A.*

albiventris species complex hereafter. See Appendix 3 for geographic distribution of the *A. albiventris* species complex.

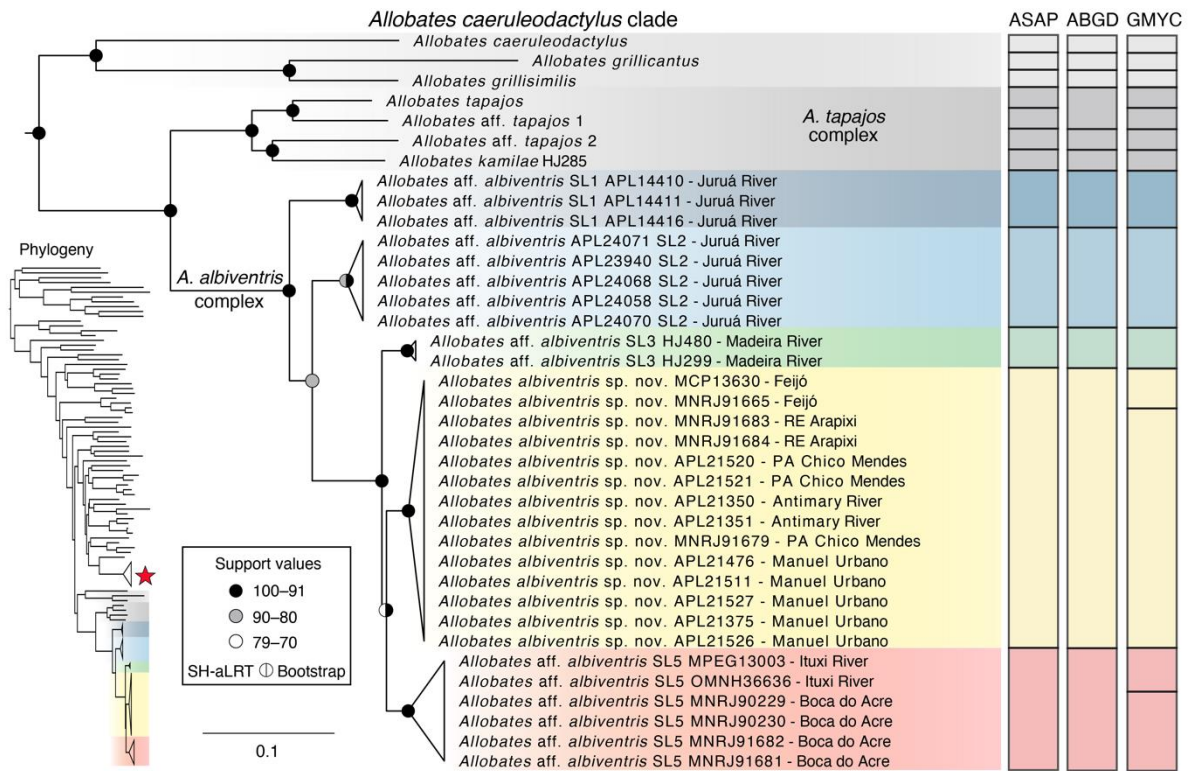


Figure 2. Phylogeny of *Allobates* based on five mitochondrial genes (12S, 16S, COI, Cytb and ND1), focusing on relationships within the *Allobates caeruleodactylus* clade. Node support was calculated following 10,000 ultrafast bootstrap approximation replicates and 10,000 replicates of the SH-aLRT branch test. Vertical bars represent species delimited with 16S through Automatic Barcode Gap Discovery (ABGD), Assemble Species by Automatic Partitioning (ASAP) and Generalized Mixed Yule Coalescent (GMYC) methods. The phylogenetic tree inset at lower left indicates the location of the *A. caeruleodactylus* clade in the overall *Allobates* phylogeny (Supplemental Figure 1). Red star in the compressed phylogeny represents *A. gasconi* sensu stricto. Note that individuals of *A. albiventris* sp. nov. and *A. aff. albiventris* were reported as *A. gasconi* in previous studies.

Within the species complex that groups species previously misidentified as *Allobates gasconi*, newly sequenced individuals of the new species (*A. albiventris* sp. nov.) cluster together in a highly supported group with individuals from Feijó, Reserva Extrativista (RE) Arapixi and Parque Ambiental (PA) Chico Mendes (Acre and Amazonas, Brazil) reported by Melo-Sampaio et al. (2018). This clade is retrieved with high support as sister to *A. aff. albiventris* SL5 from Ituxi River and Boca do Acre, which is followed by *A. aff. albiventris* SL3 from the Madeira River, *A. aff. albiventris* SL2 from lower Juruá River and finally *A. aff. albiventris* SL1 from middle Juruá River (Fig. 2).

Interspecific pairwise p-distances within the *Allobates albiventris* species complex range from 1.2 to 4.8% (Table 1). The two smallest distances are between the new species (*Allobates albiventris* sp. nov.) and *A. aff. albiventris* SL3 (1.9%; 1.6–2.1%) and between the new species and *A. aff. albiventris* SL5 (2.0%; 1.2–2.5%). Although samples of the new species are widely distributed across the Brazilian state of Acre, genetic p-distances between newly sequenced individuals of the new species and those reported by Melo-Sampaio et al. (2018) are very low (0.2%; 0.0–0.4%). The small genotypic divergence is congruent with low levels of phenotypic and bioacoustic divergence observed within the new species (see below).

Single-locus DNA delimitation

The best-ranked partition computed by ASAP ($score = 3.5$; $p = 0.001$; $w = 0.0006$) delimits all five clades within the species complex as distinct species (Fig. 2). The best ASAP threshold distance for the 16S dataset in this partition is 1%. As expected, the delimitation resulting from ABGD resembles that of ASAP and delimits the same five species (Fig. 2). On the other hand, GMYC is less conservative and delimits seven species by splitting the new species and *A. aff. albiventris* SL5 into two species each (Fig. 2). The majority rule consensus of these single-locus delimitation methods supports the delimitation of five species in the *Allobates albiventris* species complex.

Morphological analyses

The first two principal components of the Shape PCA performed with 23 morphometric measurements of *A. gasconi* sensu stricto and the new species together explain 64.6% and 63.1% of the variance of males and females, respectively (Table 2). Although the two respective shape spaces do not overlap in graphical representations of shape PC1 against PC2 (Fig. 3A, C), body shape is statistically different only in shape PC1 (ANOVAs: male, $S^2 = 2.7$, $F = 221.5$, $df = 44$, $p < .0001$; female, $S^2 = 2.2$, $F = 142.2$, $df = 22$, $p < .0001$). Width of finger III (WPF), eye–nostril distance (EN) and snout length (SL) strongly contribute to the variation of shape PC1 of both males and females (Table 2). The new species and *A. gasconi* sensu stricto also differ in the isometric size of males (ANOVA: $S^2 = 0.01$, $F = 8.3$, $df = 44$, $p = .006$), with the new species being larger (Fig. 3B).

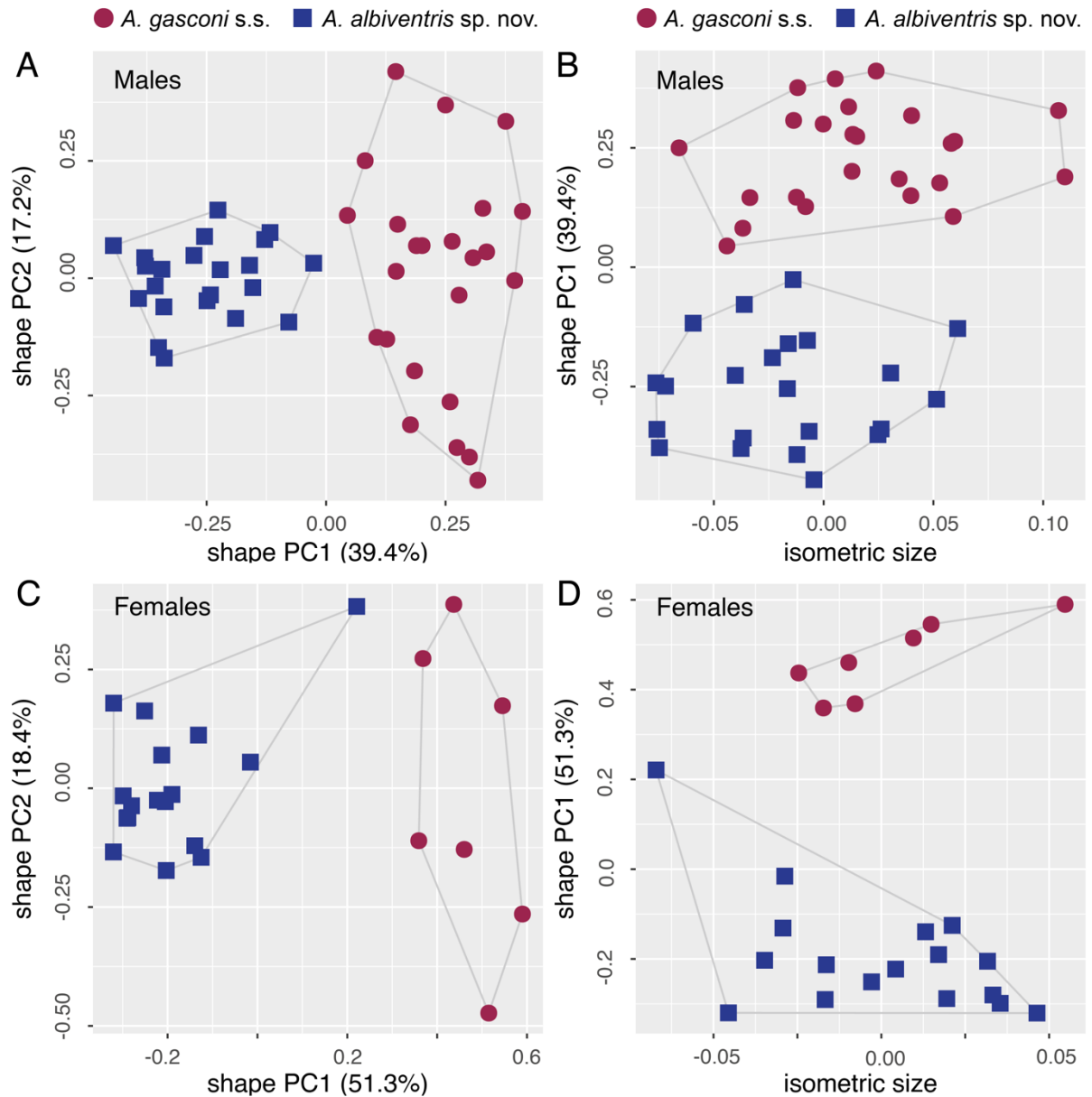


Figure 3. Shape Principal Component Analysis and isometric size of *Allobates gasconi* sensu stricto and *A. albiventris* sp. nov. Analyses are based on 23 morphometric measurements of 28 males (**A**, **C**) and 22 females (**B**, **D**). Abbreviation: s.s., sensu stricto.

Taxonomy

Allobates albiventris sp. nov.

LSID: urn:lsid:zoobank.org:act:3249CC6A-D16B-4681-8A13-7D1BDB2BDF36

Chresonymy. *Allobates gasconi*: Melo-Sampaio et al. (2018), Lima et al. (2020), Gagliardi-Urrutia et al. (2021), Jaramillo et al. (2021), Silva et al. (2022).

Allobates gasconi C: Souza et al. (2020).

Allobates gasconi SL4: Ferreira et al. (in press).

Holotype

INPAH45035 (field number APL21526), an adult male collected by J.R.D. Souza on February 5, 2020, in a forest fragment near BR 364 (08°52'27" S, 69°17'07" W, 166 masl), municipality of Manoel Urbano, state of Acre, Brazil.

Paratopotypes

Nineteen adult specimens collected by J.R.D. Souza and A.P. Lima at the same locality as the holotype: 13 males INPAH45036-40 (field numbers APL21371–75), INPAH45042-47 (field numbers 21479–80, 21511, 21518, 21525 and 21528, respectively) and MPEG 44609-10 (field number APL21370 and 21380, respectively), and 6 females INPAH45048-51 (field numbers APL21476, 21481, 21512, 21215 and 21527, respectively) and MPEG 44611-12 (field number APL21478 and 21515, respectively).

Paratypes

Twenty-three adult specimens collected between 2018 and 2020 by J.R.D. Souza in the state of Acre, and between 2015 and 2016 by P.R. Melo-Sampaio in the states of Acre and Amazonas. ACRE: 10 females MPEG44607 (field number APL21355), INPAH45052-59 (field numbers APL21357, 21523–24 and 21530–34, respectively), and MPEG44613 (field number APL21522) and 7 males INPAH45060 (field numbers APL21520–21, 21354 and 21356, respectively) MPEG44608 (field number APL21358), MPEG44606 (field number APL21352), and MNRJ 91679 (GenBank KY886578) from Parque Ambiental Chico Mendes (10°02'13"S, 67°47'36"W, 158 masl), municipality of Rio Branco; and 2 males INPAH45064-65 (field numbers APL21350–51, respectively) collected near the bank of the Antimary River (09°29'16" S, 68°21'20" W, 168 m asl.); one female (MNRJ 91665 (GenBank KY886576) and one male MCP 13630 (GenBank KY886577) from the municipality of Feijó (08°14'13" S, 70°22'44" W, 184 masl). AMAZONAS: one male MNRJ91683 (GenBank KY886574) and one female MNRJ91684 (GenBank KY886575) from Reserva Extrativista Arapixi (08°58'21" S, 67°51'50" W, 125 masl), municipality of Boca do Acre.

Referred specimen. Brazil: Acre, one male (PRMS 0360) collected by P.R. Melo-Sampaio on January 25, 2016, in Reserva Extrativista Arapixi, municipality of Boca do Acre.

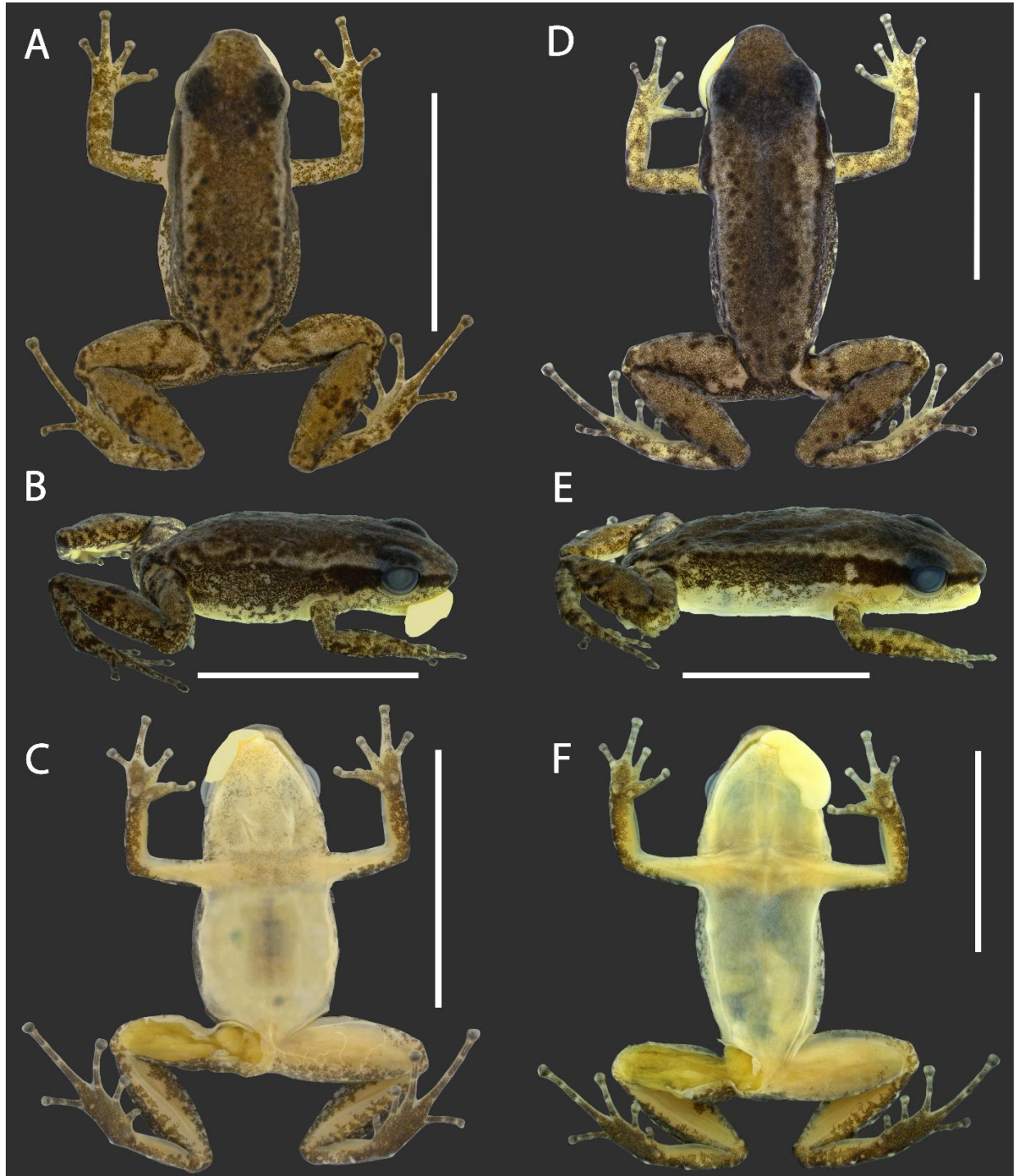


Figure 4. Dorsal, dorsolateral and ventral views of the male holotype APL21526 (A–C) and a female paratype APL21527 (D–F) of *Allobates albiventris* sp. nov. Scale bar: 10 mm. Photographs by J.R.D. Souza.

Etymology

The specific epithet *albiventris* is a combination of two Latin words, *albus* (white) and *ventris* (venter), in reference to the pale ventral coloration of the new species. Vernacular names: pale-ventered nurse frog (English), *rana cuidadora de vientre blanco* (Spanish), and *rãzinha cuidadora de ventre branco* (Portuguese).

Generic placement

The new species is allocated to the genus *Allobates* based on molecular phylogenetic analysis and phenotypic characters proposed by Grant et al. (2017): paired dorsal digital scutes (character 2), tip of finger IV reaches the distal half of distal subarticular tubercle of finger III (character 5), finger III swollen in adult males (character 21), toe IV with basal webbing and lateral fringe on its preaxial side (characters 43), pale paracloacal marks (character 50) and absence of median lingual process (character 85).

Definition

Allobates albiventris **sp. nov.** is characterized by small adult size, SVL 14.3–16.4 mm ($n = 22$) in males and 15.6–17.8 mm ($n = 16$) in females. Dorsum smooth with a high concentration of granules in the medium posterior portion. Snout semi-truncated and semi-acuminate in dorsal and lateral views; *canthus rostralis* almost straight in dorsal view; loreal region flat; nostrils visible in ventral and lateral views. Tympanum diameter 34–48% of EL. Maxillary teeth present, visible under magnification; median lingual process absent. One subarticular tubercle on finger IV; fingers II and III weakly swollen in adult males; disc on finger II approximately the same width as distal phalanx; width of discs on fingers I, III and IV represent 1.3 ± 0.3 , 1.5 ± 0.2 and 1.6 ± 0.2 of width of respective distal phalanges; tip of finger IV reaches the distal subarticular tubercle of finger III; nuptial excrescence on thumb absent; lateral keels present on fingers II–IV; supernumerary tubercles and metacarpal ridge absent; webbing absent between fingers; black gland absent on arm; tarsal keel present, tubercle-like, strongly curved towards the inner metatarsal tubercle; basal webbing present between toes II–IV, less developed between toes II and III; discs on toes II–V moderately expanded; disc on toe I not expanded, approximately the same width of distal phalanx; lateral keels present on all toes. Dorsum light brown with a dark brown band, diamond or hourglass-shaped marks; pale dorsolateral stripe present in preserved specimens, with an irregular upper border extending from the posterior region of the eyelids to the posteromedial region of the body or urostyle; pale ventrolateral

stripe absent in preservative, discontinued when present in living specimens; dark brown oblique lateral stripe present, narrower from snout to anterior corner of eye, wider from the posterior corner of the eye to the groin, with an irregular lower border. Paired dorsal digital scutes white. Cream-colored forelimbs, with scattered dark brown spots and blotches. Hind limbs light brown; anterior and dorsal portions of the thigh with dark brown spots, dorsal region light brown with scattered spots; a dark brown transverse bar present on thigh of some individuals, usually on tibia; comma-shaped, light brown to orange paracloacal mark. In life, males and females have a white belly and chest without melanophores; throat and vocal sac of males translucent white with scattered melanophores; throat white centrally and posteriorly, translucent laterally and anteriorly in females, with scattered melanophores distributed in the anterior region of the maxilla. Unpigmented intestine. Dark brown mature oocyte; unpigmented testes. Advertisement call with a duration of 42–60 ms and comprising two notes (the first note is shorter than the second), with an inter-note interval of 8–23 ms and dominant frequency of 4,953–6,331 Hz. Exotrophic tadpoles with 2 pyramidal papillae on each end of the anterior labium; 10–13 pyramidal and cylindrical papillae on the posterior labium; LTRF = 2(2)/3(1); gap in row A-2 $\approx$ 40% of A-1; relative length P-1 > P-2 > P-3; and P-3 $\approx$ 65% of P-1.

Diagnosis

Allobates albiventris **sp. nov.** differs from other *Allobates* by the following combination of characters: males in life with a throat and vocal sac translucent white with melanophores uniformly distributed and a white belly; females in life with throat white centrally and posteriorly, translucent laterally and anteriorly, chest and belly white; small adult size, SVL 14.3–16.4 mm (n = 22) in males and 15.6–17.8 mm (n = 16) in females; one subarticular tubercle on finger IV; finger III of adult males weakly swollen; disc of finger II approximately the same width as the distal phalanx; interdigital membranes present between toes II, III and IV; paired digital scutes white; advertisement call with a duration of 42–60 ms and comprising two notes (the first note is smaller than the second), with an inter-note interval of 8–23 ms and dominant frequency of 4,953–6,331 Hz; exotrophic tadpoles with 2 pyramidal papillae on each end of the anterior labium, 10–13 pyramidal and cylindrical papillae on the posterior labium, LTRF = 2(2)/3(1), gap in row A-2 $\approx$ 40% of A-1, relative length P-1 > P-2 > P-3, and P-3 $\approx$ 65% of P-1.

Comparisons

Characteristics of the compared species are presented within parentheses unless stated otherwise. Males of *Allobates albiventris* **sp. nov.** are easily distinguished from those of *A. flaviventris*, *A. fuscus*, *A. grillicantus*, *A. kamillae*, *A. melanolaemus*, *A. nidicola*, *A. ornatus*, *A. paleci*, *A. paleovarzensis*, *A. tapajos*, *A. tinae*, *A. trilineatus*, *A. vanzolinius* and *A. velocicantus* by having a translucent white throat and vocal sac in life (violaceous to gray in *A. flaviventris*, *A. pacaas* and *A. paleovarzensis*; yellow in *A. grillicantus*, *A. kamillae*, *A. paleci*, *A. tapajos* and *A. tinae*; gray to black in *A. fuscus*, *A. melanolaemus*, *A. nidicola*, *A. trilineatus* and *A. vanzolinius*; gray in *A. ornatus*; whitish centrally and yellow laterally in *A. velocicantus*). Additionally, *A. albiventris* **sp. nov.** differs from *A. pacaas* by having only one subarticular tubercle on finger IV (two tubercles); from *A. tapajos* by the presence of melanophores on the vocal sac of males (absent); from *A. flaviventris*, *A. nidicola*, *A. paleovarzensis* and *A. vanzolinius* by having a maximum SVL of 16.4 mm in males (minimum SVL 16.7 mm in *A. flaviventris*, 18.5 mm in *A. nidicola*, 18.3 mm in *A. paleovarzensis* and 21.5 mm in *A. vanzolinius*).

Males of *Allobates caeruleodactylus*, *A. conspicuus*, *A. grillisimilis*, *A. subfolionidificans* and *A. sieggreenae* have a throat and vocal sac coloration similar to *A. albiventris* **sp. nov.** However, *A. albiventris* **sp. nov.** differs from these species by the presence of dark marks or a dark brown, wide longitudinal band on the dorsum (uniform light brown dorsum in all mentioned species). In addition, *A. albiventris* **sp. nov.** differs from *A. caeruleodactylus* by having white digital scutes in life (blue); from *A. conspicuus* and *A. sieggreenae* by the absence of a continuous ventrolateral stripe (ventrolateral stripe present); from *A. grillisimilis* by having regularly distributed melanophores on the throat, vocal sac and chest (melanophores, when present, only on the jaw); and from *A. subfolionidificans* by having a dorsolateral stripe (absent) and females in life with a white chest and belly (yellow).

Although *A. albiventris* **sp. nov.** has been confused with *A. gasconi*, they are easily distinguished by the coloration of breeding adults. Males of the new species are easily distinguished from those of *A. gasconi* sensu stricto by the translucent white throat and vocal sac (gray to dark gray; Fig. 5), white chest (translucent to pinkish grey) and white belly without melanophores (belly pinkish grey anteriorly, whitish grey centrally, light or translucent grey with white and brown small blotches laterally, and yellowish or translucent grey posteriorly; Ferreira et al. in press; present study). Female *A. albiventris* **sp. nov.** have a white throat centrally and posteriorly but translucent laterally and anteriorly (light to bright yellow), a white chest (bright to whitish yellow) and a white belly (belly yellowish to whitish cream). Moreover,

finger III of adult males is only slightly swollen (moderately to highly swollen; Ferreira et al. in press; present study).



Figure 5. Coloration of the vocal sac and throat of *Allobates albiventris* **sp. nov.** (A, B) and *A. gasconi* sensu stricto (C, D). Photographs by J.R.D. Souza (A, B) and A.P. Lima (C, D).

The advertisement calls of *Allobates albiventris* **sp. nov.** differ from *A. gasconi* sensu stricto by a call duration of 50 ± 4 ms (94 ± 33 ms), exclusively composed of 2 notes (2–4 notes, mainly 3 notes) with the first note always shorter than the second (notes with similar duration) and an inter-note interval of 16 ± 4 ms (30 ± 4 ms; Ferreira et al. in press). Calls of *A. albiventris* **sp. nov.** and *A. trilineatus* are similar: both are composed of two notes and are arranged in call series. However, the former species differs from *A. trilineatus* by having a call duration of 42–60 ms (60–80 ms) and the first note always shorter and with a lower dominant frequency than the second one (similar duration and dominant frequency in both notes; Jaramillo et al. 2021). Calls of *A. albiventris* **sp. nov.** have two notes exclusively, which differs from *A. caeruleodactylus*, *A. melanolaemus*, *A. nidicola*, *A. paleovarzensis*, *A. sieggreenae*, *A.*

subfolionidificans, *A. tapajos* and *A. tinae* (calls composed of one note in each species). Moreover, calls of *A. albiventris* **sp. nov.** are commonly arranged in call series (single calls emitted regularly through time in *A. caeruleodactylus*, *A. nidicola* and *A. subfolionidificans*) with a call duration of 42–60 ms (151–507 ms in *A. grillicantus*; 122–305 ms in *A. grillisimilis*; 180–340 ms in *A. paleci*; and 1,870–2,890 ms in *A. velocicantus*) and are composed exclusively of two notes (3–15 notes in *A. grillicantus* and *A. grillisimilis*; 16–33 ms in *A. paleci*; and 66–138 in *A. velocicantus*). As in *A. albiventris* **sp. nov.**, regular calls of *A. flaviventris* are composed of two notes, but in the former species calls are emitted with a dominant frequency of 4,953–6,331 Hz (3,618–4,651 Hz in *A. flaviventris*). The advertisement calls of *A. conspicuus*, *A. fuscellus*, *A. ornatus*, *A. pacaas* and *A. vanzolinus* are unknown.

Tadpoles of *Allobates albiventris* **sp. nov.** easily differ from those of *A. gasconi* sensu stricto by having a LTRF = 2(2)/3(1) and a tail highly pigmented with brown spots of various shapes and sizes resembling a marbled pattern (LTRF = 2(2)/2(1) and tail poorly pigmented; Ferreira et al. in press); from those of *A. nidicola* by being exotrophic and having a spiracle (endotrophic, spiracle absent); from *A. subfolionidificans* and *A. tapajos* by having two pyramidal papillae on each side of the anterior labium (six in *A. subfolionidificans*; four or five in *A. tapajos*); from *A. velocicantus* by having pyramidal and cylindrical papillae on the posterior labium (only pyramidal in *A. velocicantus*); from *A. grillicantus* by having LTRF = 2(2)/3(1) [LTRF = 2(2)/3 in *A. grillicantus*]; from *A. grillisimilis* and *A. paleovarzensis* by having LTRF = P-1 > P-2 > P-3 (P-3 = P-2 = P-1 in *A. grillisimilis*; P-2 > P-1 > P-3 in *A. paleovarzensis*); from *A. caeruleodactylus* by the gap in row A-2 $\approx$ 40% of A-1 and P-3 $\approx$ 65% of P-1 (A-2 gap $\approx$ 58% of A-1 and P-3 $\approx$ 37% of P-1 in *A. caeruleodactylus*). Tadpoles of *A. conspicuus*, *A. flaviventris*, *A. fuscellus*, *A. melanolaemus*, *A. ornatus*, *A. pacaas*, *A. tinae*, *A. sieggreenae*, *A. trilineatus* and *A. vanzolinus* are unknown.

Description of the holotype

Adult male, APL21526 (Figs. 4A–C; 6A, C; 8A–C; Table 3). Snout-vent length 14.6 mm. Head wider than long (HW/HL = 1.04); HW equals 35% of SVL and HL equals 33% of SVL. Eye diameter exceeds distance from eye to nostril (EL/END = 1.33); EL equals 43% of HL. Interorbital region flat; IOD equals 88% of HW. Tympanum rounded, visible to the naked eye. Snout slightly rounded in dorsal and lateral view. Inter-nostril region flat; nostrils rounded, laterally positioned and visible in lateral and ventral view; IND equals 49% of IOD. *Canthus rostralis* straight in dorsal view; loreal region flat. Maxillary teeth present. Median lingual

process absent. Vocal sac single, subgular. Lateral folds of vocal sac present at the level of angle of maxilla.

Palmar tubercle rounded and conspicuous, diameter 0.42 mm. Thenar tubercle elliptical and conspicuous, width 0.29 mm. Diameter of thenar tubercle equals 69% of that of the palmar tubercle. Subarticular tubercles protruding, oval on finger I and rounded in other fingers; two tubercles on finger III but one in each of the others; distal tubercle smaller than proximal tubercle on finger III; tubercle on finger I larger than others. Supernumerary tubercles absent. Lateral keels on fingers I–IV, poorly defined on finger I. When placed side by side, the tip of finger IV reaches the distal subarticular tubercle of finger III. Preaxial phalangeal swelling on finger II and III. Relative length of fingers: $IV < II < I < III$. Discs are wider than the third phalanx on fingers I, III and IV, but approximately the same width on finger II. Paired dorsal digital scutes present.

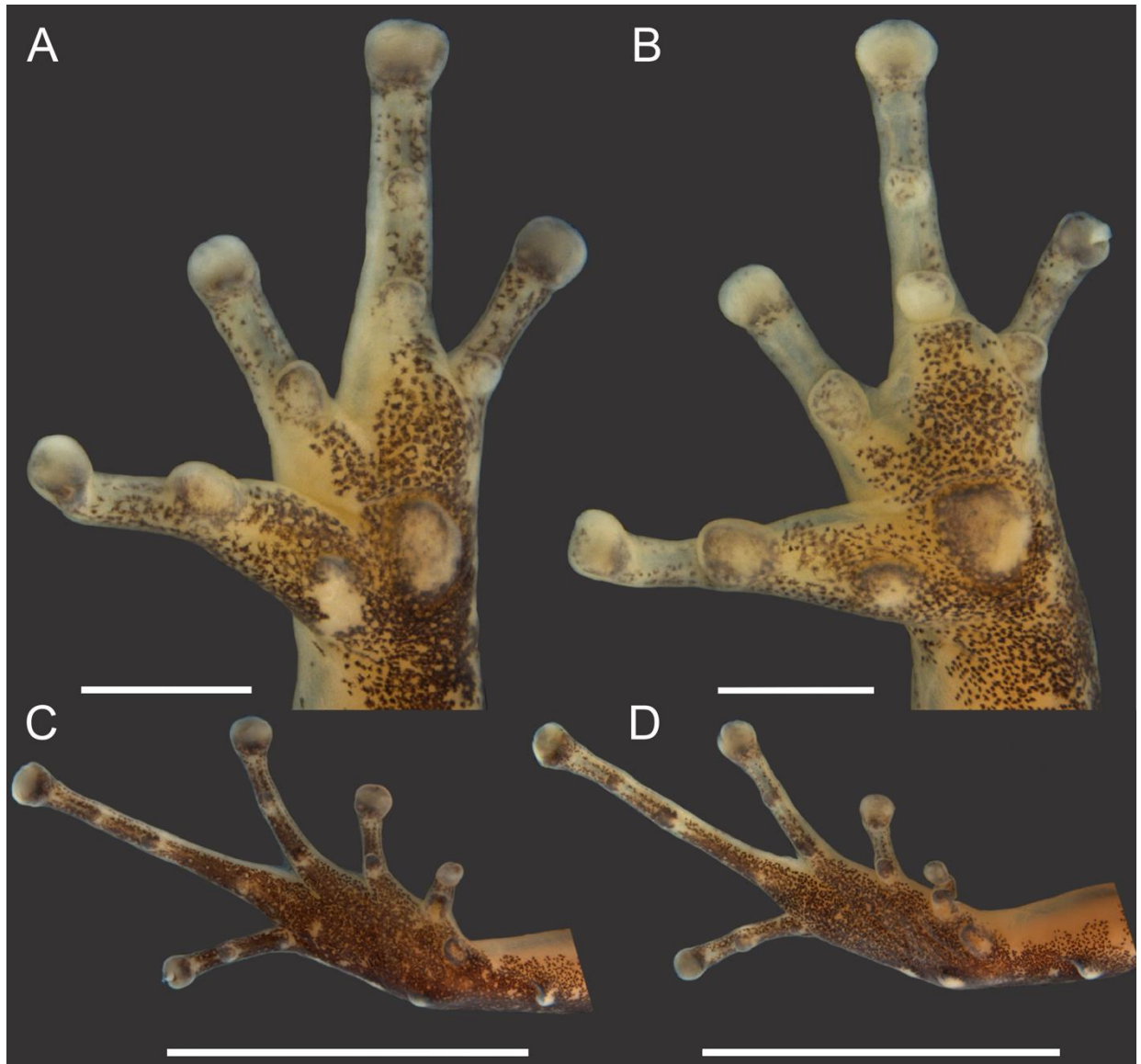


Figure 6. Ventral view of the hand (A, B) and foot (C, D) of *Allobates albiventris* **sp. nov.** (A, C) Male holotype APL21526. (B, D) Female paratype APL21527. Scale bar: 1 mm (A, B) and 5 mm (C, D). Photographs by J.R.D. Souza.

Tibia and thigh lengths approximately the same ($TL/LL = 1.01$), equal 49% and 48% of SVL, respectively. Foot length 97% of tibia length. Tarsal keel conspicuous and curved, narrowing towards the internal metatarsal tubercle. Internal metatarsal tubercle protuberant, elliptical. External metatarsal tubercle small and round, protruding, smaller than diameter of internal metatarsal tubercle. Metatarsal fold absent. Lateral keels present on preaxial and postaxial sides of each toe. Basal webbing between toes II and IV. Subarticular tubercles rounded and evident; one each on toes I and II but two each on toes III–V. Discs rounded, wider

than distal phalanx on toes II, III and IV but of similar width on toe I; disc of toe V with smaller expansion compared to toes II–IV. Paired dorsal digital scutes present.

Dorsal skin smooth with small flattened and barely visible tubercles, mostly on the posterior portion; skin on arms smooth; skin on legs smooth with small tubercles. Ventral surface of body, arms and legs smooth.

In preservative (Fig. 4A–C), dorsal surface of body and limbs light brown; numerous dark brown melanophores form a longitudinal band, which is slightly constrained behind the eyes and diffuse towards the cloaca (Fig. 4A). A narrow and cream-colored dorsolateral stripe with an irregular upper border extends from posterior region of eyelids to mid-posterior region of body (Fig. 4B). Lateral stripe dark brown; darkest from the tip of the snout to the region above the axilla; lower border regular on snout but irregular in ventrolateral region of body (Fig. 4B). Paler oblique stripe diffuse, extends from the inguinal region to the axilla. Ventrolateral stripe absent. Ventrolateral region beige; melanophores form small dark brown irregular spots. Transverse dark brown bar on thigh and tibia is widest on tibia (Fig. 4A). Anterior region of thigh light brown, posterior region dark brown; paracloacal marks cream, conspicuous. Chest, throat and vocal sac cream with small dark brown melanophores; belly cream, lacks melanophores or dark spots. Ventral surface of arms beige with scarce brown melanophores; light brown on forearms. Mid-ventral surface of thigh and tibia cream, without melanophores; ventrolateral surface with irregular dark brown spots (Fig. 4C). Palmar and plantar surfaces dark brown (Figs. 4C, 5A, C). Tongue longer than wide, with anterior third attached to the floor of the mouth, cream-colored (Fig. 4B).

Coloration in life is similar to that in preservative. Dark marks, spots, stripes, lines and bars are more conspicuous. Background coloration of the dorsum cream. Ventrolateral stripe discontinuous, from the posterior corner of the eye to the axilla; small iridescent dots and spots visible below the dark brown band (Fig. 8A–C). Iris metallic bronze, pupil black. Ventral surface of arms and legs varies between light gray and rosaceous gray. Throat and vocal sac translucent white; chest and belly white. Paired digital dorsal scutes white.

Variation in the type series

Variation in morphometric measurements of *Allobates albiventris* **sp. nov.** is summarized in Table 3. Sexual dimorphism is present in SVL and 12 body ratios. Females are larger than males (SVL; $S^2 = 10.3$, $F = 50.48$, $df = 36$, $\text{adj } p = .0002$) but smaller in EN ($S^2 = 0.0006$, $F = 8.46$, $df = 33$, $\text{adj } p = .0152$), LL ($S^2 = 0.0028$, $F = 20.94$, $df = 33$, $\text{adj } p = .0004$), TL ($S^2 = 0.0073$,

$F = 11.64$, $df = 36$, $\text{adj } p = .0062$), FL ($S^2 = 0.0025$, $F = 8.31$, $df = 36$, $\text{adj } p = .0152$), UAL ($S^2 = .0010$, $F = 7.55$, $df = 36$, $\text{adj } p = 0.0195$), FAL ($S^2 = 0.0006$, $F = 6.58$, $df = 33$, $\text{adj } p = .0266$), HANDI ($S^2 = 0.0005$, $F = 8.87$, $df = 36$, $\text{adj } p = .0148$), HANDII ($S^2 = 0.0004$, $F = 9.33$, $df = 36$, $\text{adj } p = .0139$), HANDIV ($S^2 = 0.0005$, $F = 7.09$, $df = 36$, $\text{adj } p = .0220$), WPF ($S^2 = 0.00003$, $F = 42.28$, $df = 27$, $\text{adj } p < .0001$), WTT ($S^2 = 0.00002$, $F = 38.79$, $df = 27$, $\text{adj } p < .0001$) and EL ($S^2 = 0.0003$, $F = 13.55$, $df = 36$, $\text{adj } p = .0035$).

Unlike the holotype, a third subarticular tubercle is present on the proximal portion of toe IV in 53% of the rest of the type series (12 males and 8 females). When present, it is approximately half the size of other subarticular tubercles on the same toe (Fig. 7G, H).

In preservative, dorsal coloration of the type series ranges from light to dark brown (Fig. 7). Dark hourglass-like markings are present in 45% of males ($n = 10$) and 44% of females ($n = 7$) and are more noticeable in individuals with lighter background coloration (e.g., Fig. 7B, C). In the other specimens, a light-to-dark-brown band extends down the center of the dorsum from the interorbital region to the urostyle. The light dorsolateral line is present in all males and females. However, its variable thickness is more evident in specimens lacking hourglass markings (Fig. 7E). Conspicuous dark transverse bars on the tibia, which are barely visible or absent on the thigh, are present in 71% of specimens (12 males and 15 females). Both males and females lack visible melanophores on the belly (Fig. 7D, F) but the sexes differ with respect to the presence and concentration of melanophores on the chest and throat; melanophores are present on the chest in 72% of males ($n = 16$) but only 31% of females ($n = 5$). Although melanophores are present on the throat of all individuals, females have fewer of them and they are distributed mainly in the anterior and peripheral region of the throat, while males have more of them and are distributed uniformly.

As in the holotype, coloration in life is similar to that in preservative (Fig. 7). The lateral brown stripe is darkest from the tip of the snout to the region above the axilla, becoming lighter and more diffuse as it extends to the inguinal region (Fig. 8A, E, H).

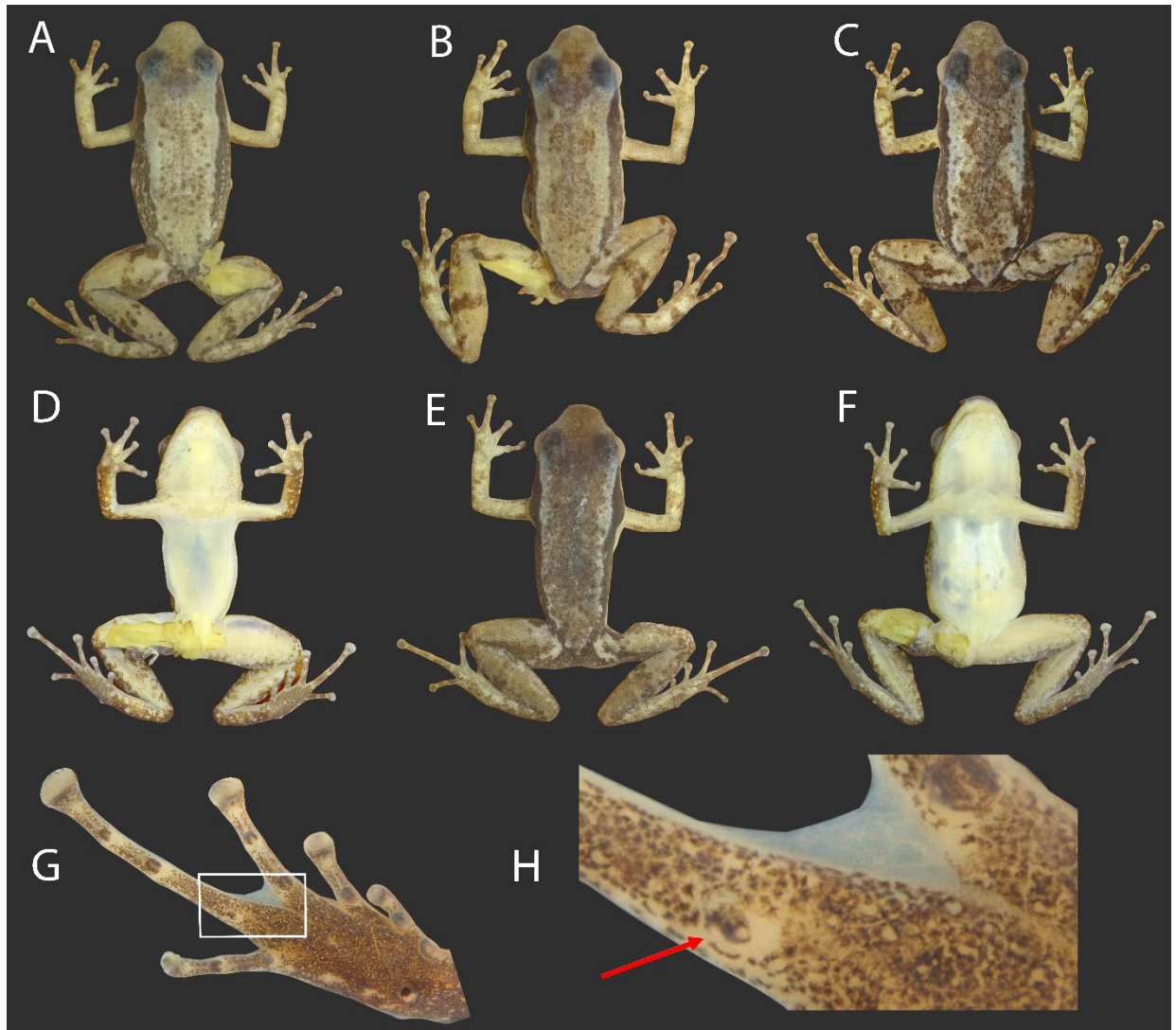


Figure 7. Paratypes of *Allobates albiventris* **sp. nov.** (A) Female, APL21515, SVL 16.2 mm. (B) Male, APL21373, SVL 14.7 mm. (C, F) Female, APL21524, SVL 17.0 mm. (D) Male, APL21520, SVL 15.6 mm. (E) Male, APL21525, SVL 14.9 mm. (G, H) Male, APL21350, SVL 16.4 mm. White rectangle in (G) delimits the magnified region (H) illustrating the third tubercle on toe IV (arrow). Photographs by J.R.D. Souza.



Figure 8. Coloration in life of *Allobates albiventris* sp. nov. from Manoel Urbano and Rio Branco, state of Acre, Brazil. (A–C) Male holotype, SVL 14.6 mm; (D–F) Female, APL21527, SVL 16.8 mm; (G–I) Male, MNRJ 91679, SVL 16.0 mm; (J–L) Female, APL21522, SVL 16.0 mm; (M) Male, uncollected; (N) Male, APL21511, SVL 15.3 mm; (O) Male, uncollected. Photographs by J.R.D. Souza (A–F, J–O) and P.R. Melo-Sampaio (G–I).

Advertisement call

The advertisement call of *Allobates albiventris* **sp. nov.** is formed by a pair of notes that is emitted singly or in a series of as many as 16 calls. Calls emitted in series of one, two, three, four or five calls correspond to 88% of recorded call arrangements (Fig. 9A–E). Calls have an average duration of 50 ± 4 ms (42–60 ms). The first note of each call is always shorter (13 ± 2 ms; 8–20 ms) than the second one (20 ± 2 ms; 14–26 ms) (Fig. 10B), and the inter-note interval ranges from 8 to 23 ms (16 ± 4 ms). Call series with two calls have a duration of 220–610 ms (320 ± 70 ms), while series with three, four or five calls have a duration of 370–1,030 ms (600 ± 110 ms), 580–1,420 ms (950 ± 170 ms) and 790–1,700 ms ($1,260 \pm 200$ ms), respectively. The inter-call interval within call series is 130–390 ms (210 ± 50 ms); the interval between call series is 410–4,400 ms ($1,010 \pm 670$ ms). Notes have modulated frequencies, ascending from beginning to end (Fig. 10B–E). The first note has a slightly lower dominant frequency ($5,409 \pm 211$ Hz; 4,952–5,857 Hz) than the second note ($5,849 \pm 250$ Hz; 5,189–6,331 Hz). Lower and upper frequency values are presented in Table 4.

Eggs and larvae

Descriptions of quantitative characters of tadpoles are based on six specimens at Gosner stage 34. Morphometric measurements are presented in Table 5. Body ovoid in dorsal view, ellipsoid in lateral view (Fig. 10A–C). Body length (BL) 31–33% of total length (TL) and tail length 67–69% of TL; body wider than tall (BH 50–65% of BW) and longer than wide (BW 61–80% of BL); HWLE 72–93% of BW; snout rounded in dorsal and lateral view; END 60–78% of ED; eyes directed dorsally and laterally; IOD 26–33% of HWLE. Nostrils located dorsolaterally and directed anterolaterally, visible in dorsal and lateral view; inter-nostril distance 38–44% of HWLE. Fleshy ring present on inner margin of nostrils, round, not ornamented. Spiracle single, sinistral, tubular, 0.46–0.67 mm long; it is attached laterally, a little below half body length and just dorsal to the intestine. Gut coiled. Vent tube dextral, 1.23–1.60 mm long. Dorsal fin arises around 2 mm from the junction of tail with body, shallow edge anteriorly, maximum height at mid posterior region of tail (Fig. 10B). Dorsal fin higher than ventral fin. Tail tip acuminate, not flagellated. Maximum tail height 2.44–2.69 mm. Width of tail musculature 35–50% of body width; height of tail musculature 59–77% of body height.

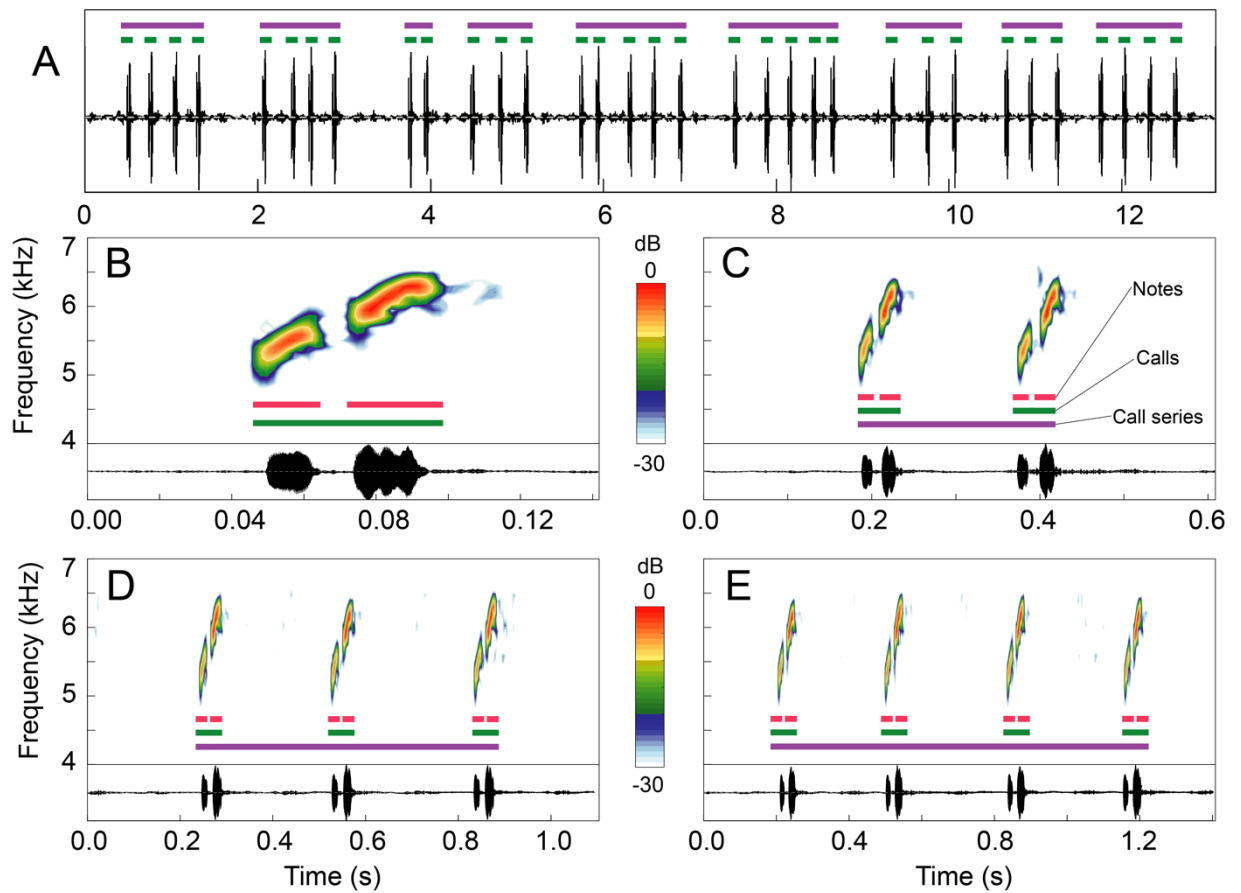


Figure 9. Advertisement calls of the holotype of *Allobates albiventris* **sp. nov.** (APL21526, FNJV 53687). (A) Oscillogram showing the continuous emission of calls with variable arrangements. Spectrograms and oscillograms of the four most common call arrangements: single call (B) and series of two (C), three (D) and four calls (D). Note that each call is composed of two notes. Air temperature: 22°C. Abbreviations: dB, decibels; kHz, kilohertz; s, second.

Oral disc positioned anteroventrally, laterally emarginate, oval in ventral view (Fig. 10B, C), 1.44–1.93 mm wide, and corresponding to 37–49% of body width at height of spiracle. Anterior labium with four short pyramidal papillae distributed in a single row and two papillae at each end of the labium (Fig. 10D, E). Posterior labium with a single row of 10–13 papillae, of which one or two are pyramidal at each end and the rest are cylindrical. Cylindrical papillae vary in size along the labium, being shorter at the ends and medially (Fig. 10D, E).

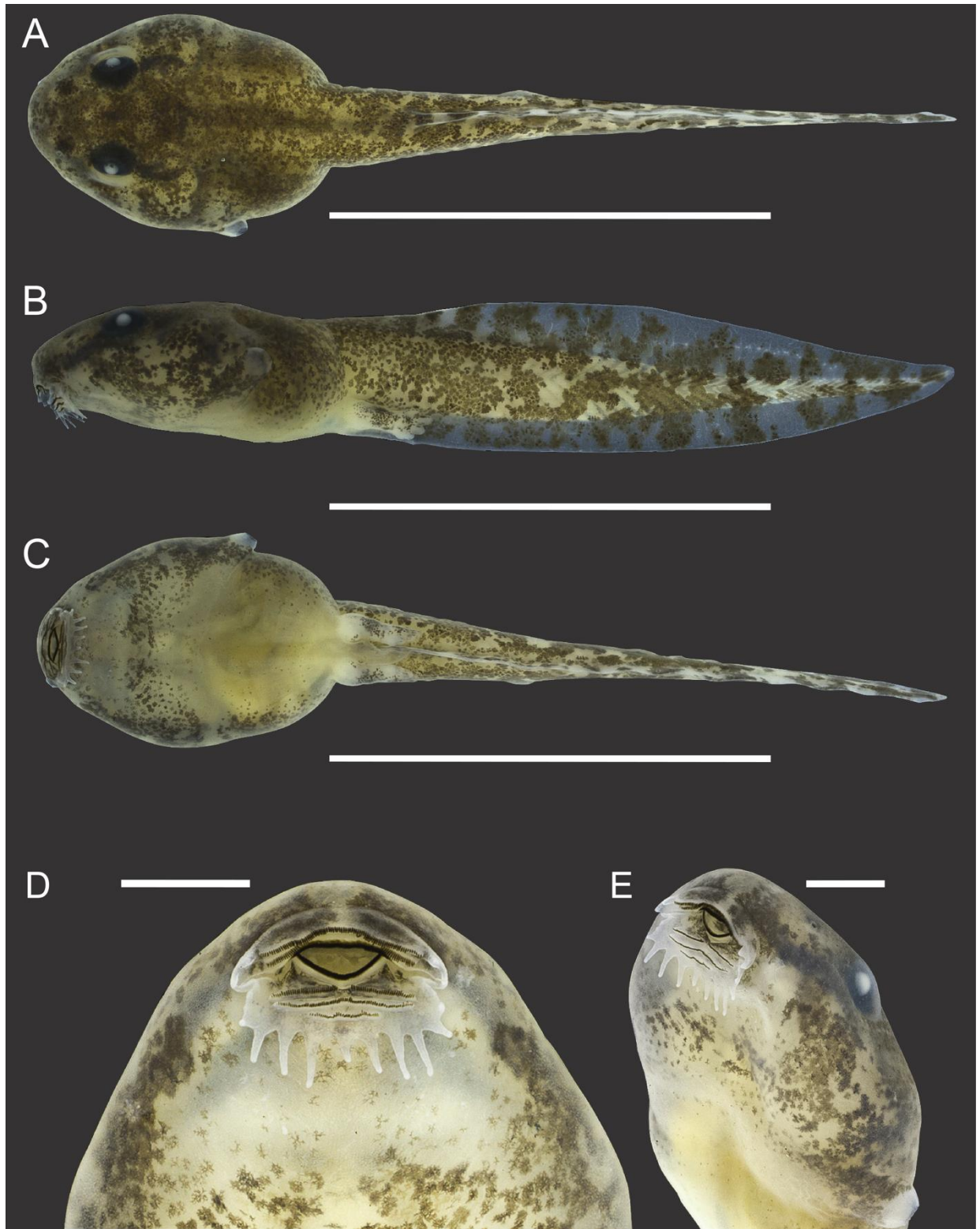


Figure 10. Preserved tadpole of *Allobates albiventris* sp. nov. (lot APL21518b), Gosner stage 34. (A) Dorsal, (B) lateral and (C) ventrolateral views of body, and (D) ventral and (E) ventrolateral views of oral disc. Scale bar: 10 mm (A–C) and 1 mm (D, E). Photographs by J.R.D. Souza.

Submarginal papillae absent. Upper jaw sheath arch-shaped, longer than lower jaw sheath and without median cut. Lower jaw V-shaped. Sheaths with serrations along their entire length. Labial formula of the keratodont row (LTRF) is 2(2)/3(1). Row A-1 measures 1.16 ± 0.05 mm; A-2 measures 1.07 ± 0.04 mm but is interrupted in the central region by a gap of approximately 40%. Row P-1 measures 1.02 ± 0.11 mm, with a small medial gap. Rows P-2 and P-3 are complete; they measure 0.98 ± 0.08 mm and 0.67 ± 0.14 mm, respectively (Fig. 10D).

In preservative, body and tail muscles assume different shades of cream, with brown melanophores forming blotches and spots of various shapes and sizes. Fins cream but translucent, with brown spots of various shapes and sizes resembling a marbled pattern. Venter cream but translucent, with thickened brown melanophores mainly in the central region. Internal organs are visible through the skin (Fig. 10C).

Geographic distribution and natural history

Allobates albiventris **sp. nov.** is known from only five localities in southwestern Brazilian Amazonia: four in the state of Acre and one in the state of Amazonas (Figs. 1 and 11). The species inhabits the leaf litter of primary and secondary ombrophilous forests at elevations between 125 and 184 masl (Fig. 11). It has a diurnal habit, generally active between 0500 and 1800 h, with vocal activity peaks between 0500–0900 h and 1600–1800 h.

Allobates albiventris **sp. nov.** breeds in the rainy season between November and March. Males vocalize both on litterfall and while perched on shrubs or fallen branches up to 40 cm above ground (Fig. 8M). Clutches are deposited on the adaxial portion of living, attached leaves of small shrubs approximately 10–15 cm from the ground (Fig. 12F–H). We found eight egg clutches—three at the type locality (Manoel Urbano, Acre), four in Parque Ambiental Chico Mendes (Rio Branco, Acre) and one in Reserva Extrativista Arapixi (Boca do Acre, Amazonas). The number of eggs per clutch ranged from 17 to 31 (Fig. 12F, G). Two clutches were found on the same leaf (nest) (Fig. 12H). In freshly laid eggs, approximately half of the animal pole is darkly pigmented; the rest of the egg is white. The eggs are surrounded by an opaque, colloidal gel (Fig. 12F, G), which becomes denser and more opaque over the course of larval development.

Four mating pairs were observed in courtship, one at the type locality and three in Parque Ambiental Chico Mendes. Each observation began with the approach of a female to the perch where a male was emitting courtship calls. In each case, the male, perceiving the approach of a female, began to emit courtship calls interspersed with advertisement calls. He then jumped

from the call perch and attempted to guide the female (Fig. 12B) by conducting her to an oviposition site (bushes or seedlings) located up to 3 m from the perch. During the courtship march (*sensu* Rocha et al. 2018), which lasted between 3 and 5 min, the male continued to emit advertisement and courtship calls while the female sporadically made short stops. Once arriving at the oviposition site, the male jumped to the adaxial surface of the leaf, located 10–15 cm from the ground, and continued vocalizing. The female followed the male and positioned herself underneath the leaf, at the edge closest to the ground. She then observed the male, raising her head toward the leaf. In all courtships, females positioned themselves vertically, with forelimbs only lightly touching the ground, for up to 1 min before jumping to the leaf (Fig. 12C). After the jump, the female approached the male. On one occasion, the female faced the male and put her snout on the male's pectoral region, then turned in the opposite direction. The male then climbed onto the female's back and they initiated amplexus (it was not possible to clearly discern the type of amplexus). In the other courtships, the female approached the male and quickly was grabbed by him. The male positioned himself laterally, snout to snout, and with one hand held the female's head, either by the region between the eyes and nostrils or directly on the snout (Fig. 12D). The resulting cephalic amplexus lasted from 1 to 4 min. During and after amplexus, a barely audible vocalization (similar to “cheeps”), was emitted by the male. Following amplexus, on one occasion the male quickly jumped into the leaf litter and returned to vocalize within 3 min. In the three other courtships, the male remained on the leaf while the female deposited eggs (Fig. 12E, H) but left the leaf before she finished. Two males initiated advertisement calls while the female was still ovipositing (Fig. 12H), while the third left the nest without vocalizing. On two occasions oviposition started with the male still in amplexus.

During oviposition, females repeatedly moved their heads upwards. This movement was interspersed with continuous clockwise or counterclockwise rotations relative to the vertical plane. Oviposition lasted ~11 to 15 min and ended when the female stopped the tilting motion with her head. However, she remained at the nest, on the clutch, and performing sporadic turns (apparently, hydrating the clutch, as her skin became excessively moist). Residence time of each female after oviposition ranged from 10 to 15 min, and the total time in the nest from 21 to 30 min. Males returned to the nest between 25 and 30 min after the female left, probably to hydrate the eggs and promote swelling of the investing jelly coats. We collected one clutch immediately after the female's departure, prior to the male's return, and the embryos developed normally. Only one male was observed performing larval transport (Fig. 12I).

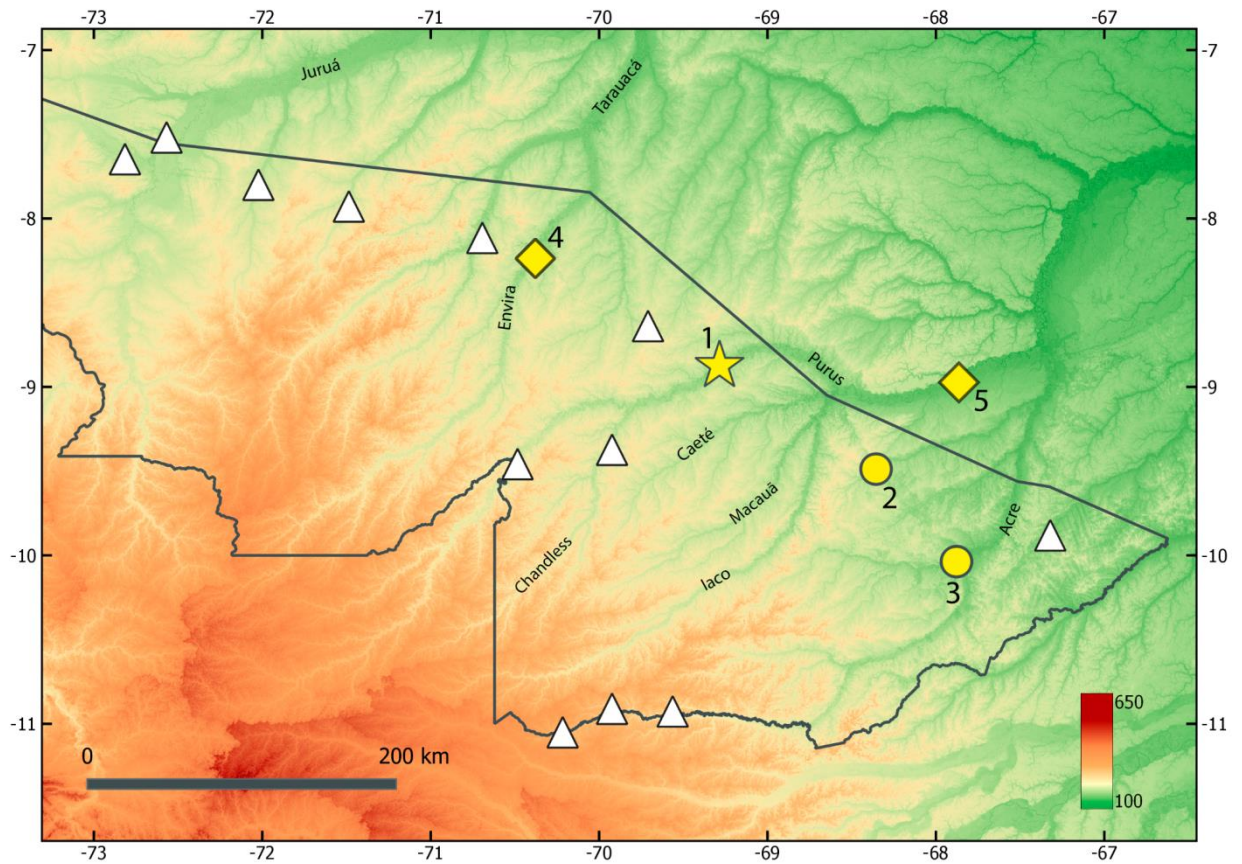


Figure 11. Geographic distribution of *Allobates albiventris* **sp. nov.** Star = type locality (1, Manoel Urbano); circles = additional paratype localities (2, Antimary River; 3, Parque Ambiental Chico Mendes); and rhombus (4, Feijó; 5, Reserva Extrativista Arapixi). White triangles denote sampled localities where the new species was not recorded.



Figure 12. Habitat and natural history of *Allobates albiventris* sp. nov. (A) Typical understory of open ombrophilous forest at the type locality in Manoel Urbano, state of Acre, Brazil. (B) Beginning of courtship behavior, in which a male is leading a female to an oviposition site. (C) Female (unvouchered) positioning herself to jump to the adaxial surface of the leaf, where the male is vocalizing. (D) Mating pair (unvouchered) in cephalic amplexus. (E) Mating pair (unvouchered) on the leaf, with the female in oviposition and the male silent. (F) Recently deposited clutch, with the smallest clutch recorded (17 eggs). (G) The largest clutch recorded (31 eggs). (H) Mating pair on a leaf, where a male vocalizes while a female deposits a second clutch on the same leaf. (I) Male APL21518 carrying 10 tadpoles on his back. Photographs by J.R.D. Souza.

Discussion

Nearly ten years ago, Simões et al. (2013b) reported that nurse frogs from middle Juruá and Ituxi rivers attributed to *Allobates gasconi* likely represent independent evolutionary lineages,

a claim that was later corroborated by Vacher et al. (2020) through molecular delimitation methods. However, the absence of topotypical genetic and bioacoustic data for *A. gasconi* sensu stricto hampered the subsequent taxonomic study of related taxa (Melo-Sampaio et al. 2018). Recently, Ferreira et al. (in press) showed based on topotypical data that *A. gasconi* sensu stricto is not closely related to several populations previously assigned to it. *Allobates albiventris* is the first nominal species of this complex to be named and described. Since the name *A. gasconi* is not applicable to any population in this species complex, we instead refer to it as the *A. albiventris* species complex. The number of candidate species associated with *A. albiventris* differs among phylogenetic studies (Vacher et al. 2020; Réjaud et al. 2020; Ferreira et al. in press; present study). However, molecular delimitation as presented here represents an initial attempt to delimit species, and we emphasize the need to further integrate morphologic, genetic and bioacoustic data to fully evaluate the cryptic diversity associated with *A. albiventris*.

Allobates albiventris is known from five lowland localities in southwestern Brazilian Amazonia. The species inhabits open ombrophilous forests dominated by bamboos of the genus *Guadua* and occupies pristine as well as secondary forests with low to moderate levels of anthropization (e.g., Parque Ambiental Chico Mendes and Reserva Extrativista Arapixi). Twelve additional localities, all at elevations above 300 masl, were sampled in the upper Juruá and Purus river basins, but *A. albiventris* was only found below 200 masl. We also surveyed seven permanent sampling modules along the upper Madeira River, all < 200 masl in open ombrophilous forests dominated by palms, but we found only *A. aff. albiventris (gasconi)* SL3 (Vacher et al. 2020). The geographic distribution of *A. albiventris* seems bounded on the east by the Acre River and on the west by the Envira River. Geographic distribution of other candidates in the *A. albiventris* complex remains poorly known since each is currently known from one or two localities (Appendix 3); further sampling in southwestern and central Amazonia is needed to clarify the range of each candidate and the drivers limiting it.

Réjaud et al. (2020) suggest that modern large rivers promoted diversification of *Allobates* after the end of the Pebas system and that meandering rivers in western Brazilian Amazonia (e.g., Juruá and Purus rivers) may have played a modest role as biogeographic barriers, especially with respect to the diversification of early diverging species. Although the divergence time between *A. aff. gasconi* SL3 and the *A. tapajos* species complex is estimated at ~ 9.4 Mya (Réjaud et al. 2020), the divergence time among lineages of the *A. albiventris* species complex remains unknown. As this species complex is endemic to southwestern Amazonian lowlands and its distribution seems limited by small rivers and altitude, further

biogeographic studies focused on its diversification may clarify the role of historical and geographical drivers in the speciation of this relatively ancient Amazonian frog complex.

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Competing interests

The authors declare that they have no competing interests.

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Appendix 1. Species, museum voucher specimens and GenBank accession numbers of sequences used in molecular analyses. Accession numbers in bold font denote sequences generated in the present study.

<i>Species</i>	Voucher	12S	16S	ND1	COI	Cytb
<i>Allobates</i> aff. <i>granti</i>	125PG	JN690205	JN690931			
<i>A.</i> aff. <i>magnussoni</i>	977126	MT627173	MT627173	MT627173	MT627173	MT627173
<i>A.</i> aff. <i>melanolaemus</i>	MTR28013	MT627203	MT627203	MT627203	MT627203	MT627203
<i>A.</i> aff. <i>olfersioides</i> 1	MTR17821		KDQF01003353			
<i>A.</i> aff. <i>olfersioides</i> 2	MTR16435	MT627202	MT627202	MT627202	MT627202	MT627202
<i>A.</i> aff. <i>olfersioides</i> 3	JFT959		KDQF01002701			
<i>A.</i> aff. <i>tapajos</i> 1	MTR10084	MT627197	MT627197	MT627197	MT627197	MT627197
<i>A.</i> aff. <i>tapajos</i> 2	AF1906	MT627175	MT627175	MT627175	MT627175	MT627175
<i>A.</i> aff. <i>tinae</i> 1	MPEG13397	DQ502213	DQ502213		DQ502900	DQ502648
<i>A.</i> aff. <i>trilineatus</i> 1	FGZC3247	MT627185	MT627185	MT627185	MT627185	MT627185
<i>A.</i> aff. <i>trilineatus</i> 2	JMP2313	MT627195	MT627195	MT627195	MT627195	MT627195
<i>A.</i> aff. <i>undulatus</i>	AMNHA159139	DQ283044	DQ283044		DQ502756	DQ502459
<i>A. algorei</i>	TNHCFS5551	HQ290950	HQ290950	HQ290950		HQ290530
<i>A. amissibilis</i>	PK3798	MT627204	MT627204	MT627204	MT627204	MT627204
<i>A. bacurau</i>	INPAH35401		KU195701			
<i>A. caeruleodactylus</i>	MTR10227	MT627199	MT627199	MT627199	MT627199	MT627199
<i>A. caldwellae</i>	MPEG13826	DQ502099	DQ502099			DQ502531
<i>A. carajas</i>	BM163	MT627183	MT627183	MT627183	MT627183	MT627183
<i>A. chalcopis</i>	Alca1	MT627182	MT627182	MT627182	MT627182	
<i>A. albiventris</i> sp. nov.	MCP13630		KY886577			KY886618
<i>A. albiventris</i> sp. nov.	MNRJ91665		KY886576			KY886617
<i>A. albiventris</i> sp. nov.	MNRJ91679		KY886578			KY886619
<i>A. albiventris</i> sp. nov.	MNRJ91683		KY886574			KY886615
<i>A. albiventris</i> sp. nov.	MNRJ91684		KY886575			KY886616

<i>A. albiventris</i> sp. nov.	APL21521	ON937753			
<i>A. albiventris</i> sp. nov.	APL21520	ON937752			
<i>A. albiventris</i> sp. nov.	APL21350	ON937746			
<i>A. albiventris</i> sp. nov.	APL21351	ON937745			
<i>A. albiventris</i> sp. nov.	APL21476	ON937748			
<i>A. albiventris</i> sp. nov.	APL21511	ON937749			
<i>A. albiventris</i> sp. nov.	APL21526	ON937750			
<i>A. albiventris</i> sp. nov.	APL21527	ON937751			
<i>A. albiventris</i> sp. nov.	APL21375	ON937747			
<i>A. conspicuus/subfolionidificans</i>	FGZC3279	MT627186	MT627186	MT627186	MT627186
<i>A. crombiei</i>	AF1097	MT627174	MT627174	MT627174	MT627174
<i>A. femoralis</i>	AF3224	MT627179	MT627179	MT627179	MT627179
<i>A. femoralis</i> SS	AfemShucv3a	DQ523001	DQ523072		DQ523142
<i>A. flaviventris</i>	HJ545	MT627192	MT627192	MT627192	MT627192
<i>A. fratisenesescus</i>	QCAZ54377		MF624172		MF614174
<i>A. gasconi</i> sensu stricto	GGU684		MT524137		
<i>A. gasconi</i> sensu stricto	APL23345		ON997555		
<i>A. gasconi</i> sensu stricto	APL23342		ON997554		
<i>A. gasconi</i> sensu stricto	APL23346		ON997547		
<i>A. gasconi</i> sensu stricto	APL23354		ON997552		
<i>A. gasconi</i> sensu stricto	APL23411		ON997558		
<i>A. aff. albiventris</i> SL1	APL14410		KJ747333		
<i>A. aff. albiventris</i> SL1	APL14411		KJ747334		
<i>A. aff. albiventris</i> SL1	APL14416		KJ747335		
<i>A. aff. albiventris</i> SL2	APL23940		OQ297604		
<i>A. aff. albiventris</i> SL2	APL24058		OQ297605		
<i>A. aff. albiventris</i> SL2	APL24068		OQ297606		

<i>A. aff. albiventris</i> SL2	APL24070		OQ297607			
<i>A. aff. albiventris</i> SL2	APL24071		OQ297608			
<i>A. aff. albiventris</i> SL3	HJ480	MT627191	MT627191	MT627191	MT627191	MT627191
<i>A. aff. albiventris</i> SL3	HJ299		KDQF01002640			
<i>A. aff. albiventris</i> SL5	MNRJ 91681		KY886572			
<i>A. aff. albiventris</i> SL5	MNRJ 91682		KY886573			KY886614
<i>A. aff. albiventris</i> SL5	OMNH36636	DQ502209	DQ502209		DQ502898	DQ502644
<i>A. aff. albiventris</i> SL5	MPEG13003	DQ502052	DQ502052		DQ502777	DQ502483
<i>A. aff. albiventris</i> SL5	MNRJ 90229		KY886570			KY886612
<i>A. aff. albiventris</i> SL5	MNRJ 90230		KY886571			KY886613
<i>A. goianus</i>	SAMA8574	MT627207	MT627207	MT627207	MT627207	MT627207
<i>A. granti</i>	AF1998	MT627176	MT627176	MT627176	MT627176	MT627176
<i>A. grillicantus</i>	MPEG43046		MW220039			
<i>A. grillisimilis</i>	MTR12749	MT627200	MT627200	MT627200	MT627200	MT627200
<i>A. hodli</i>	ABU2194		KX044279			
<i>A. humilis/pittieri</i>	CVULA5690	KJ940454	KJ940454			
<i>A. insperatus/juami</i>	JMP1703	MT627193	MT627193	MT627193	MT627193	MT627193
<i>A. juanii/ranoides</i>	ARA2394	DQ502271	DQ502271		DQ502933	DQ502702
<i>A. kamilae</i>	HJ285	MT627189	MT627189	MT627189	MT627189	MT627189
<i>A. kingsburyi</i>	QCAZ16523	AY364549	HQ290963			HQ290541
<i>A. magnussoni</i>	BM168	MT627184	MT627184	MT627184	MT627184	MT627184
<i>A. marchesianus</i>	AJC2498	MT627180	MT627180	MT627180	MT627180	MT627180
<i>A. masniger</i>	MTR10155	MT627198	MT627198	MT627198	MT627198	MT627198
<i>A. melanolaemus</i>	NMP6V711404		MT524148			
<i>A. nidicola</i>	MPEG13821	DQ502101	DQ502101			DQ502533
<i>A. niputidea</i>	MUJ3520	DQ502272	DQ502272		DQ502934	DQ502703
<i>A. nunciatus</i>	MPEG36777	MT627196	MT627196	MT627196	MT627196	MT627196

<i>A. olfersioides</i>	MNRJ79897	MF624178	MF624178			MF614175
<i>A. ornatus</i>	MHNSM22863		EU342550			
<i>A. pacaas</i>	MZUSP158938		MT076999			
<i>A. paleovarzensis</i>	JMP2196	MT627194	MT627194	MT627194	MT627194	
<i>A. sieggreenae</i>	MCP14533		MW293942			
<i>A. sp. Huanuco</i>	FGZC3348	MT627187	MT627187	MT627187	MT627187	MT627187
<i>A. sp. Neblina</i>	MTR15537	MT627201	MT627201	MT627201	MT627201	MT627201
<i>A. sumtuosus</i>	AF2212	MT627177	MT627177	MT627177	MT627177	MT627177
<i>A. talamancae</i>	QCAZ35236	MT627205	MT627205	MT627205	MT627205	MT627205
<i>A. Tapajos</i>	MJH3973	DQ502110	DQ502110		DQ502820	DQ502542
<i>A. tinae</i>	HJ298	MT627190	MT627190	MT627190	MT627190	MT627190
<i>A. trilineatus</i>	AF4493		MT524111			
<i>A. undulatus</i>	AJC3040	MT627181	MT627181	MT627181	MT627181	MT627181
<i>A. velocicantus</i>	MCP10187/88	MF624181	MF624181			MF614178
<i>A. zaparo</i>	USNM546405	DQ502026	DQ502026		DQ502752	DQ502455
<i>Ameerega hahneli</i>	AF2673	MT627178	MT627178	MT627178	MT627178	MT627178
<i>Anomaloglossus stepheni</i>	MJH3928	DQ502107	DQ502107		DQ502818	DQ502539
<i>Aromobates saltuensis/nocturnus</i>	TNHCFS5541/AMNHA130042	HQ290970	HQ290970	HQ290970	DQ502860	DQ502592
<i>Colostethus brachistriatus</i>	CZPDUV4603	MF624204	MF624204		MF614304	MF614198
<i>Dendrobates auratus</i>	MVZHerp149723	JX564862	JX564862	JX564862	JX564862	JX564862
<i>Epipedobates boulengeri</i>	UMMZ227952/QCAZ16574	HQ290997	HQ290997	HQ290997	DQ502742	DQ502447
<i>Leucostethus fugax</i>	QCAZ16513	HQ290958	HQ290958	HQ290958		HQ290538
<i>Mannophryne collaris</i>	FS5523	MT627188	MT627188	MT627188	MT627188	MT627188
<i>Phyllobates terribilis</i>	TNHC64420/AMNHA118566	HQ291006	HQ291006	HQ291006	DQ502861	DQ502593
<i>Rheobates palmatus</i>	RHEOPALM	MT627206	MT627206	MT627206	MT627206	MT627206
<i>Silverstoneia nubicola/erasmios</i>	TNHCFS4942/MAR336	HQ290966	HQ290966	HQ290966	MF614333	MF614237

Appendix 2. Specimens examined. Acronyms: **INPAH**, Herpetological Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil; **MNRJ**, Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil; **MPEG**, Herpetological Collection of the Museu Paraense Emílio Goeldi, Belém, Pará, Brazil; **MZUSP**, Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil. **UFAC-RB**, Herpetological Collection of the Universidade Federal do Acre, Rio Branco, Acre, Brazil.

Allobates caeruleodactylus. Adults. Brazil: Amazonas: km 12 on the road to Autazes (INPAH 7238 [holotype]; 7229–32, 7234–37 [paratypes]). Tadpoles. Brazil: Amazonas: km 12 on the road to Autazes [lots INPAH 8037–46, 8085].

Allobates flaviventris. Adults. Brazil: Acre: Senador Guimard, Fazenda Bonal (UFAC-RB 4650 [holotype], 4599–4601, 4603, 4631–35, 4675–77 [paratypes]).

Allobates fuscillus. Adults. Brazil: Amazonas: Juruá River: Penedo (INPAH 2532 [holotype], 2312, 2333, 2351, 2531, 2534, 2537 [paratypes]), Jainu (INPAH 3114, 3127, 3250, 3270, 3514 [paratypes]).

Allobates gasconi. Adults. Brazil: Amazonas: Jainu, west bank of Juruá River (INPAH 3082 [holotype]; 3079, 3083–84, 3090, 3093, 3249, 3412, 3483, 3491, 3494, 3512–13 [paratypes]).

Allobates grillicantus. Adults. Brazil: Pará: Trairão (MPEG 43046 [holotype]; 43038–41, 43045, 43047–50 [paratopotypes]); km 24 on Brazilian federal highway BR-163 (INPAH 41352–56 [paratypes]). Tadpoles. Brazil: Pará: Trairão (lots MPEG 43051–53, INPAH 41357).

Allobates grillisimilis. Adults. Brazil: Amazonas: Borba (INPAH 30779 [holotype]; 30780–808 [paratopotypes]); Nova Olinda do Norte (INPAH 30809–23 [paratypes]).

Allobates nidicola. Adults. Brazil: Amazonas: km 12 on road to Autazes (INPAH 8093 [holotype]; 7253–59, 7261–62, 8094 [paratypes]; 28122, 28124, 28127, 28129, 28131, 28144, 28159, 28163, 28166, 28169, 28171–72, 28174, 28179, 28184–85 [topotypes]). Tadpoles. Brazil: Amazonas: km 12 on road to Autazes (lots INPAH 8021–33, 8137–39).

Allobates pacaas. Adults. Brazil: Rondônia: Parque Nacional dos Pacaás Novos (MZUSP 158934 [holotype], 158935–38, MNRJ 93128–29, MPEG 42818–19, UFAC-RB 9470 [paratypes]).

Allobates paleovarzensis. Adults. Brazil: Amazonas: Careiro da Várzea (INPAH 20904 [holotype]; 20861–903, 20905 [paratypes]).

Allobates subfolionidificans. Adults. Brazil: Acre: Rio Branco, Parque Zoobotânico da Universidade Federal do Acre (INPA-H 13760 [holotype], 11959–74, 13749–54 [paratypes]). Tadpoles. Brazil: Acre: Rio Branco, Parque Zoobotânico da Universidade Federal do Acre (lots INPAH 14822, 14823).

Allobates tapajos. Adults. Brazil: Pará: Parque Nacional da Amazônia (INPAH 34425 [holotype]; 34402–24 [paratypes]). Tadpoles. Brazil: Pará: Parque Nacional da Amazônia (lots INPAH 34426–27).

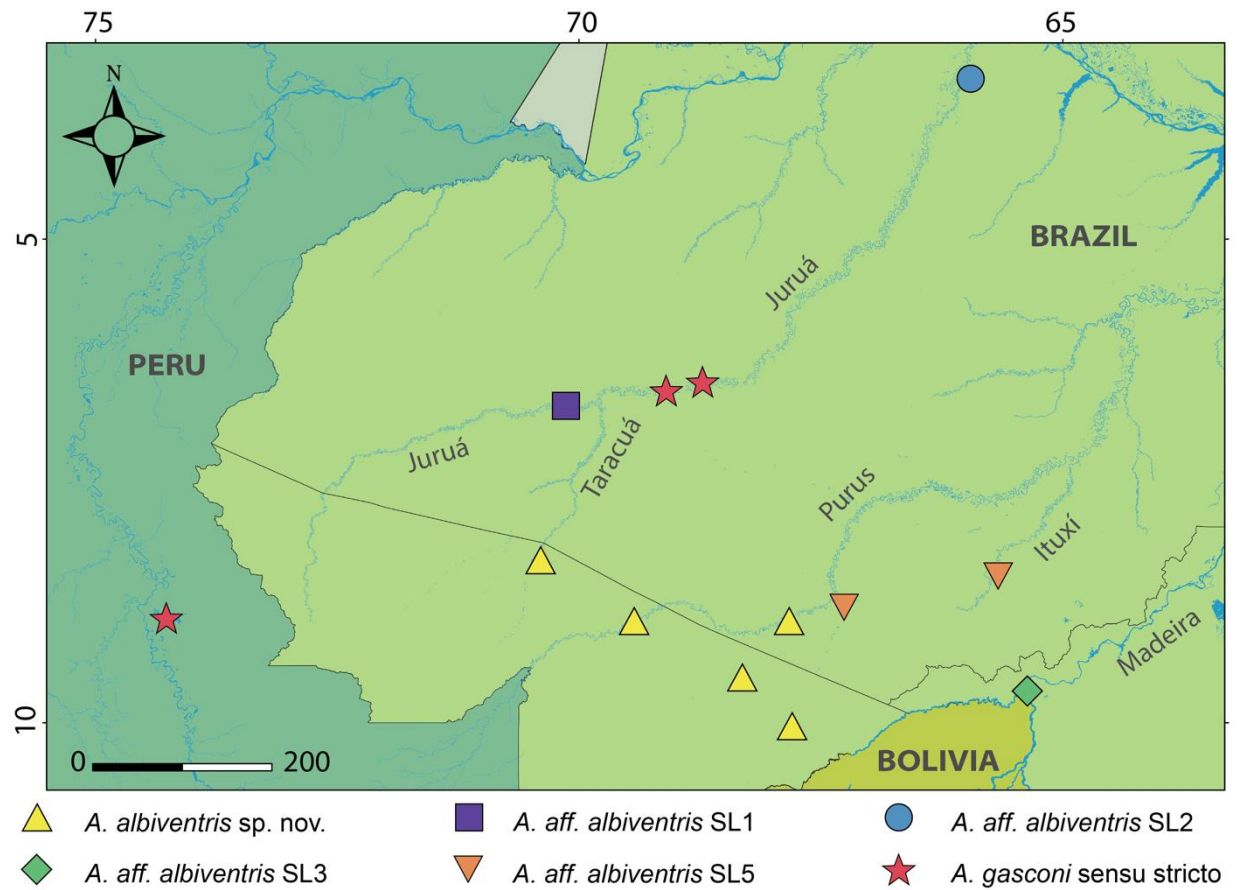
Allobates tinae. Adults. Brazil: Rondônia: Porto Velho, west bank of upper Madeira River (INPAH 41012–21, 41029–36, 41041–44); Amazonas: Boca do Acre (INPAH 40976, 41022, 41027, 41037, 41040; MNRJ 90214 [holotype], 90215–28, [paratypes]; UFAC-RB 4625 [paratype]); Acre: Senador Guimard (UFAC-RB 4604, 4636–37 [paratypes]).

Allobates trilineatus. Adults. Brazil: Acre: Rio Branco, Parque Zoobotânico of Universidade Federal do Acre (INPAH 11958–93)

Allobates vanzolinius. Adults. Brazil: Amazonas: Juruá River, Vai-Quem-Quer (INPAH 4896 [holotype]; 4903–05, 4912 [paratypes]); Juruá, Rio Juruá (INPAH 3381, 3413 [paratypes]).

Allobates velocicantus. Adults. Brazil: Acre: Mâncio Lima (INPAH 41342 [holotype], 41338–41, 41344–48 [paratypes]). Tadpoles. Brazil: Acre: Mâncio Lima (lot INPAH 41351).

Appendix 3. Geographic distribution of samples of *Allobates gasconi* and *A. albiventris* species complex included in the phylogenetic analyses. Lineage numbers follow those of Figure 2.



TABLES

Table 1. Pairwise genetic distances between and within *Allobates gasconi* sensu stricto (SS) and species of the *Allobates albiventris* species complex based on a fragment of 16S rRNA. Uncorrected pairwise distances (p-distance) and Kimura 2-parameter distances (K2P) are below and above the diagonal, respectively. Numbers in bold along the diagonal denote intraspecific p-distances. Distances are expressed as percentage and presented as mean and range.

Species	1	2	3	4	5	6
1 <i>A. gasconi</i> SS	0.4 (0.0–0.9)	10.2 (9.0–12.1)	9.6 (8.8–11.0)	10.2 (9.0–11.8)	10.9 (9.5–12.3)	10.7 (9.3–12.6)
2 <i>A. aff. albiventris</i> SL1	9.5 (8.4–11.1)	0.1 (0.0–0.2)	3.1 (2.8–3.3)	4.8 (4.6–5.0)	4.1 (3.8–5.0)	4.5 (4.0–5.0)
3 <i>A. aff. albiventris</i> SL2	9.0 (8.3–10.1)	3.0 (2.8–3.2)	0.2 (0.0–0.6)	3.8 (3.7–4.1)	3.3 (2.8–4.3)	3.9 (3.5–4.4)
4 <i>A. aff. albiventris</i> SL3	9.5 (8.5–10.8)	4.6 (4.4–4.8)	3.7 (3.6–3.9)	0.2 (0.0–0.4)	1.9 (1.6–2.2)	2.3 (1.7–2.9)
5 <i>A. albiventris</i> sp. nov.	10.0 (8.8–11.0)	4.0 (3.8–4.8)	3.2 (2.9–4.1)	1.9 (1.6–2.1)	0.2 (0.0–0.4)	2.0 (1.2–2.5)
6 <i>A. aff. albiventris</i> SL5	9.9 (8.7–11.4)	4.3 (3.8–4.8)	3.7 (3.4–4.2)	2.2 (1.7–2.8)	2.0 (1.2–2.4)	0.6 (0.0–1.6)

Table 2. Loadings of morphometric variables on the first two shape principal components (PC) based on 28 male and 22 female *Allobates gasconi* sensu stricto and *Allobates albiventris* **sp. nov.** Numbers in bold denote variables that make the highest contributions to each shape PC. Measurement acronyms are described in the text; n, sample size.

Variables	Males		Females	
	shape.PC1	shape.PC2	shape.PC1	shape.PC2
DPT	-0.047	-0.203	-0.088	-0.067
EL	-0.043	0.029	0.048	0.071
EN	0.415	-0.008	0.335	-0.026
FAL	0.073	0.037	0.047	0.171
FL	0.137	0.147	0.115	0.071
HANDI	0.037	0.056	0.083	0.158
HANDII	0.02	0.11	0.051	0.118
HANDIII	0.082	0.037	0.052	0.075
HANDIV	0.038	0.103	0.06	0.091
HL	-0.197	0.112	-0.172	0.104
HW	-0.135	0.133	-0.161	0.126
IN	0.082	0.007	0.032	0.07
IO	-0.026	0.101	-0.029	0.097
SL	0.406	0.088	0.345	0.051
SVL	0.044	0.026	0.039	0.054
THL	0.03	0.142	0.016	0.083
TL	0.035	0.204	0.014	0.072
TYM	-0.161	-0.07	-0.081	-0.04
UAL	0.002	0.118	0.003	0.015
WFD	-0.231	0.033	-0.154	-0.123
WPF	-0.617	-0.213	-0.775	-0.158
WTD	-0.187	-0.147	0.029	-0.124
WTT	0.247	-0.843	0.191	-0.887

Table 3. Morphometrics measurements in millimeters of *Allobates albiventris* **sp. nov.** and *A. gasconi* sensu stricto. Values represent mean $\pm$ standard deviation (range). Measurement acronyms are described in the text; n, sample size.

Characters	<i>Allobates albiventris</i> sp. nov.			<i>Allobates gasconi</i> sensu stricto	
	Holotype	Males (n = 21)	Females (n = 16)	Males (n = 24)	Females (n = 7)
SVL	14.6	15.3 $\pm$ 0.5 (14.3–16.4)	16.5 $\pm$ 0.5 (15.6–17.8)	16.2 $\pm$ 0.6 (14.8–17.1)	16.8 $\pm$ 0.8 (16.0–18.0)
IOD	4.5	4.6 $\pm$ 0.2 (4.3–5.0)	4.8 $\pm$ 0.2 (4.5–5.1)	4.7 $\pm$ 0.3 (4.3–5.5)	4.7 $\pm$ 0.2 (4.3–4.9)
HW	5.1	5.1 $\pm$ 0.2 (4.8–5.5)	5.5 $\pm$ 0.2 (5.1–5.9)	4.9 $\pm$ 0.3 (4.3–6.0)	4.9 $\pm$ 0.2 (4.5–5.1)
HL	4.9	4.9 $\pm$ 0.2 (4.6–5.4)	5.3 $\pm$ 0.2 (4.9–5.8)	4.6 $\pm$ 0.4 (4.0–5.7)	4.7 $\pm$ 0.3 (4.3–4.9)
IND	2.2	2.2 $\pm$ 0.1 (2.0–2.5)	2.4 $\pm$ 0.1 (2.3–2.6)	2.4 $\pm$ 0.1 (2.2–2.7)	2.5 $\pm$ 0.1 (2.3–2.6)
LL	7.0	7.0 $\pm$ 0.4 (6.4–8.0)	7.3 $\pm$ 0.2 (7.0–7.9)	7.4 $\pm$ 0.6 (6.5–8.8)	7.4 $\pm$ 0.3 (7.0–7.8)
TL	7.1	7.3 $\pm$ 0.4 (6.7–8.1)	7.6 $\pm$ 0.2 (7.4–8.0)	7.7 $\pm$ 0.6 (6.6–9.2)	7.7 $\pm$ 0.4 (7.2–8.1)
FL	6.9	6.7 $\pm$ 0.4 (6.1–7.7)	6.9 $\pm$ 0.3 (6.6–7.3)	7.4 $\pm$ 0.5 (6.6–8.9)	7.5 $\pm$ 0.3 (7.0–8.1)
UAL	3.6	3.8 $\pm$ 0.2 (3.5–4.2)	4.0 $\pm$ 0.2 (3.6–4.3)	4.0 $\pm$ 0.2 (3.5–4.5)	3.9 $\pm$ 0.2 (3.8–4.4)
FAL	3.3	3.4 $\pm$ 0.2 (3.1–3.8)	3.6 $\pm$ 0.5 (3.3–5.6)	3.6 $\pm$ 0.3 (2.9–4.4)	3.7 $\pm$ 0.3 (3.5–4.3)
HANDI	2.6	2.7 $\pm$ 0.1 (2.5–2.9)	2.8 $\pm$ 0.1 (2.6–3.0)	2.9 $\pm$ 0.2 (2.4–3.3)	3.0 $\pm$ 0.2 (2.8–3.2)
HANDII	2.5	2.6 $\pm$ 0.1 (2.4–2.8)	2.7 $\pm$ 0.1 (2.5–2.9)	2.7 $\pm$ 0.2 (2.4–3.1)	2.8 $\pm$ 0.1 (2.6–3.0)
HANDIII	3.5	3.6 $\pm$ 0.2 (3.2–4.0)	3.8 $\pm$ 0.1 (3.5–4.1)	3.9 $\pm$ 0.3 (3.4–4.4)	4.0 $\pm$ 0.2 (3.8–4.2)
HANDIV	2.3	2.4 $\pm$ 0.2 (2.1–2.7)	2.5 $\pm$ 0.1 (2.4–2.7)	2.6 $\pm$ 0.3 (2.0–3.1)	2.7 $\pm$ 0.1 (2.5–2.8)
WFD	0.5	0.5 $\pm$ 0.1 (0.4–0.6)	0.6 $\pm$ 0.0 (0.5–0.7)	0.5 $\pm$ 0.1 (0.4–0.6)	0.5 $\pm$ 0.0 (0.5–0.6)
WPF	0.3	0.3 $\pm$ 0.0 (0.2–0.4)	0.3 $\pm$ 0.0 (0.3–0.4)	0.4 $\pm$ 0.1 (0.3–0.5)	0.3 $\pm$ 0.0 (0.3–0.4)
DPT	0.5	0.5 $\pm$ 0.1 (0.5–0.6)	0.6 $\pm$ 0.0 (0.5–0.7)	0.6 $\pm$ 0.1 (0.4–0.8)	0.5 $\pm$ 0.0 (0.5–0.6)
WTT	0.4	0.4 $\pm$ 0.0 (0.3–0.4)	0.4 $\pm$ 0.0 (0.3–0.5)	0.5 $\pm$ 0.1 (0.3–0.7)	0.5 $\pm$ 0.1 (0.3–0.7)
WTD	0.6	0.7 $\pm$ 0.1 (0.5–0.8)	0.7 $\pm$ 0.1 (0.6–0.8)	0.6 $\pm$ 0.1 (0.5–0.7)	0.7 $\pm$ 0.1 (0.6–0.8)
TYM	0.8	0.9 $\pm$ 0.1 (0.7–1.1)	0.9 $\pm$ 0.1 (0.9–1.1)	0.8 $\pm$ 0.1 (0.6–0.9)	0.9 $\pm$ 0.1 (0.6–0.9)
EL	2.0	2.1 $\pm$ 0.1 (2.0–2.4)	2.2 $\pm$ 0.1 (2.1–2.4)	2.1 $\pm$ 0.1 (1.9–2.4)	2.3 $\pm$ 0.1 (2.1–2.5)
END	1.5	1.4 $\pm$ 0.1 (1.2–1.8)	1.5 $\pm$ 0.1 (1.4–1.7)	1.9 $\pm$ 0.2 (1.4–2.1)	1.9 $\pm$ 0.1 (1.8–2.0)

SL	2.2	2.0 ± 0.2 (1.7–2.4)	2.1 ± 0.1 (1.9–2.4)	2.5 ± 0.2 (2.1–2.9)	2.7 ± 0.1 (2.5–2.8)
<i>Allobates albiventris</i> sp. nov.			<i>Allobates gasconi</i> sensu stricto		
Characters	Holotype	Males (n = 21)	Females (n = 16)	Males (n = 24)	Females (n = 7)
SVL	14.6	15.3 ± 0.5 (14.3–16.4)	16.5 ± 0.5 (15.6–17.8)	16.2 ± 0.6 (14.8–17.1)	16.8 ± 0.8 (16.0–18.0)
IOD	4.5	4.6 ± 0.2 (4.3–5.0)	4.8 ± 0.2 (4.5–5.1)	4.7 ± 0.3 (4.3–5.5)	4.7 ± 0.2 (4.3–4.9)
HW	5.1	5.1 ± 0.2 (4.8–5.5)	5.5 ± 0.2 (5.1–5.9)	4.9 ± 0.3 (4.3–6.0)	4.9 ± 0.2 (4.5–5.1)
HL	4.9	4.9 ± 0.2 (4.6–5.4)	5.3 ± 0.2 (4.9–5.8)	4.6 ± 0.4 (4.0–5.7)	4.7 ± 0.3 (4.3–4.9)
IND	2.2	2.2 ± 0.1 (2.0–2.5)	2.4 ± 0.1 (2.3–2.6)	2.4 ± 0.1 (2.2–2.7)	2.5 ± 0.1 (2.3–2.6)
LL	7.0	7.0 ± 0.4 (6.4–8.0)	7.3 ± 0.2 (7.0–7.9)	7.4 ± 0.6 (6.5–8.8)	7.4 ± 0.3 (7.0–7.8)
TL	7.1	7.3 ± 0.4 (6.7–8.1)	7.6 ± 0.2 (7.4–8.0)	7.7 ± 0.6 (6.6–9.2)	7.7 ± 0.4 (7.2–8.1)
FL	6.9	6.7 ± 0.4 (6.1–7.7)	6.9 ± 0.3 (6.6–7.3)	7.4 ± 0.5 (6.6–8.9)	7.5 ± 0.3 (7.0–8.1)
UAL	3.6	3.8 ± 0.2 (3.5–4.2)	4.0 ± 0.2 (3.6–4.3)	4.0 ± 0.2 (3.5–4.5)	3.9 ± 0.2 (3.8–4.4)
FAL	3.3	3.4 ± 0.2 (3.1–3.8)	3.6 ± 0.5 (3.3–5.6)	3.6 ± 0.3 (2.9–4.4)	3.7 ± 0.3 (3.5–4.3)
HANDI	2.6	2.7 ± 0.1 (2.5–2.9)	2.8 ± 0.1 (2.6–3.0)	2.9 ± 0.2 (2.4–3.3)	3.0 ± 0.2 (2.8–3.2)
HANDII	2.5	2.6 ± 0.1 (2.4–2.8)	2.7 ± 0.1 (2.5–2.9)	2.7 ± 0.2 (2.4–3.1)	2.8 ± 0.1 (2.6–3.0)
HANDIII	3.5	3.6 ± 0.2 (3.2–4.0)	3.8 ± 0.1 (3.5–4.1)	3.9 ± 0.3 (3.4–4.4)	4.0 ± 0.2 (3.8–4.2)
HANDIV	2.3	2.4 ± 0.2 (2.1–2.7)	2.5 ± 0.1 (2.4–2.7)	2.6 ± 0.3 (2.0–3.1)	2.7 ± 0.1 (2.5–2.8)
WFD	0.5	0.5 ± 0.1 (0.4–0.6)	0.6 ± 0.0 (0.5–0.7)	0.5 ± 0.1 (0.4–0.6)	0.5 ± 0.0 (0.5–0.6)
WPF	0.3	0.3 ± 0.0 (0.2–0.4)	0.3 ± 0.0 (0.3–0.4)	0.4 ± 0.1 (0.3–0.5)	0.3 ± 0.0 (0.3–0.4)
DPT	0.5	0.5 ± 0.1 (0.5–0.6)	0.6 ± 0.0 (0.5–0.7)	0.6 ± 0.1 (0.4–0.8)	0.5 ± 0.0 (0.5–0.6)
WTT	0.4	0.4 ± 0.0 (0.3–0.4)	0.4 ± 0.0 (0.3–0.5)	0.5 ± 0.1 (0.3–0.7)	0.5 ± 0.1 (0.3–0.7)
WTD	0.6	0.7 ± 0.1 (0.5–0.8)	0.7 ± 0.1 (0.6–0.8)	0.6 ± 0.1 (0.5–0.7)	0.7 ± 0.1 (0.6–0.8)
TYM	0.8	0.9 ± 0.1 (0.7–1.1)	0.9 ± 0.1 (0.9–1.1)	0.8 ± 0.1 (0.6–0.9)	0.9 ± 0.1 (0.6–0.9)
EL	2.0	2.1 ± 0.1 (2.0–2.4)	2.2 ± 0.1 (2.1–2.4)	2.1 ± 0.1 (1.9–2.4)	2.3 ± 0.1 (2.1–2.5)
END	1.5	1.4 ± 0.1 (1.2–1.8)	1.5 ± 0.1 (1.4–1.7)	1.9 ± 0.2 (1.4–2.1)	1.9 ± 0.1 (1.8–2.0)
SL	2.2	2.0 ± 0.2 (1.7–2.4)	2.1 ± 0.1 (1.9–2.4)	2.5 ± 0.2 (2.1–2.9)	2.7 ± 0.1 (2.5–2.8)

Table 4. Advertisement call parameters of *Allobates albiventris* **sp. nov.** Values represent the mean for each calling male. Call parameters are described in the text. Localities: ANT, Antimary River; FEI, Feijó; MUR, Manoel Urbano; PCM, Parque Ambiental Chico Mendes; REA, Reserva Extrativista Arapixi. Abbreviations: AT, air temperature in degrees Celsius; FNJV, call voucher recordings deposited in the Fonoteca Neotropical Jacques Vielliard; SD, standard deviation.

FNJV	Locality	AT	CSD	ICSI	CD	ICI	ND 1	INI	ND2	LFN1	HFN1	DFN1	LFN2	HFN2	DFN2
Unvouchered	FEI	NA	480	2,010	49	160	12	19	18	5,015	5,608	5,336	5,361	5,831	5,607
Unvouchered	PCM	NA	1,040	1,190	58	240	13	20	24	4,893	5,523	5,222	5,263	5,739	5,487
53688	PCM	23.0	1,560	1,210	52	210	11	21	20	5,448	6,048	5,792	5,875	6,336	6,141
53689	PCM	23.5	700	1,680	52	230	10	22	20	4,823	5,362	5,065	5,118	5,617	5,381
Unvouchered	REA	28.0	940	1,230	49	280	10	20	18	5,374	6,008	5,705	5,695	6,222	5,991
53692	REA	28.0	440	620	47	200	11	17	20	5,181	5,944	5,606	5,685	6,273	6,002
53693	REA	29.0	360	760	48	210	13	16	18	5,023	5,714	5,400	5,522	6,006	5,768
53680	ANT	25.0	790	920	48	210	12	16	20	4,739	5,213	5,017	5,276	5,658	5,476
53681	ANT	24.5	1,050	690	51	240	13	16	21	5,09	5,673	5,416	5,548	6,083	5,861
53682	MUR	24.0	670	640	49	230	15	14	19	5,194	5,621	5,405	5,619	6,070	5,840
53685	MUR	22.0	1,400	2,120	48	220	13	14	20	5,312	5,858	5,618	5,702	6,307	6,083
53686	MUR	22.0	650	730	45	200	13	11	21	5,352	5,941	5,591	5,995	6,507	6,294
53687	MUR	22.0	780	660	45	230	13	12	20	5,098	5,663	5,392	5,709	6,267	6,036
53684	MUR	22.0	630	880	47	200	16	11	20	5,179	5,73	5,465	5,702	6,176	5,978
53683	MUR	22.0	610	850	51	230	16	15	21	5,044	5,523	5,237	5,519	6,033	5,810
53691	MUR	22.0	440	560	45	160	13	12	21	5,084	5,638	5,353	5,568	6,082	5,825
53690	MUR	21.0	550	950	50	240	12	17	20	5,036	5,493	5,278	5,568	6,020	5,805
Mean	–	23.9	700	1,010	50	210	13	16	20	5,120	5,687	5,409	5,577	6,076	5,849
SD	–	2.6	650	670	4	50	2	4	2	206	228	211	233	247	250
Min	–	21.0	40	410	42	130	8	8	14	4,640	5,153	4,953	4,909	5,413	5,189

Max	–	29.0	4,470	4,400	60	390	20	23	26	5,576	6,127	5,857	6,195	6,552	6,331
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Table 5. Morphometric measurements in millimeters of 12 tadpoles of *Allobates albiventris* sp. nov., Gosner stages 34–37, from Manoel Urbano, Acre, Brazil. Values depict mean $\pm$ standard deviation (range). Trait acronyms are defined in the text; n, sample size.

Traits	Stage 34 (n = 6)	Stage 35 (n = 1)	Stage 36 (n = 3)	Stage 37 (n = 2)
TL	17.14 $\pm$ 0.48 (16.55–17.81)	17.72	19.26 $\pm$ 0.13 (19.15–19.40)	20.03 $\pm$ 0.06 (19.99–20.08)
TAL	11.61 $\pm$ 0.38 (11.26–12.26)	12.10	13.13 $\pm$ 0.05 (13.10–13.19)	13.69 $\pm$ 0.00 (13.69–13.69)
BL	5.53 $\pm$ 0.19 (5.29–5.80)	5.63	6.13 $\pm$ 0.08 (6.05–6.22)	6.34 $\pm$ 0.06 (6.30–6.38)
BW	3.85 $\pm$ 0.36 (3.36–4.28)	4.12	3.53 $\pm$ 0.08 (3.44–3.61)	4.03 $\pm$ 0.24 (3.86–4.20)
BH	2.23 $\pm$ 0.19 (1.93–2.44)	2.18	2.27 $\pm$ 0.15 (2.10–2.35)	2.60 $\pm$ 0.12 (2.52–2.69)
HWLE	3.19 $\pm$ 0.21 (3.02–3.53)	3.70	3.02 $\pm$ 0.00 (3.02–3.02)	3.40 $\pm$ 0.06 (3.36–3.44)
TMW	1.55 $\pm$ 0.09 (1.43–1.68)	1.43	1.51 $\pm$ 0.08 (1.43–1.60)	1.64 $\pm$ 0.18 (1.51–1.76)
MTH	2.58 $\pm$ 0.09 (2.44–2.69)	2.69	2.80 $\pm$ 0.10 (2.69–2.86)	2.94 $\pm$ 0.12 (2.86–3.02)
TMH	1.54 $\pm$ 0.10 (1.43–1.68)	1.51	1.62 $\pm$ 0.05 (1.60–1.68)	1.68 $\pm$ 0.00 (1.68–1.68)
IOD	0.99 $\pm$ 0.35 (0.92–1.05)	1.01	1.04 $\pm$ 0.05 (1.02–1.09)	1.13 $\pm$ 0.06 (1.09–1.18)
IND	1.33 $\pm$ 0.06 (1.26–1.43)	1.43	1.48 $\pm$ 0.13 (1.34–1.60)	1.47 $\pm$ 0.06 (1.43–1.51)
END	0.57 $\pm$ 0.04 (0.50–0.63)	0.55	0.52 $\pm$ 0.02 (0.50–0.55)	0.59 $\pm$ 0.00 (0.59–0.59)
NSD	0.52 $\pm$ 0.07 (0.42–0.63)	0.50	0.67 $\pm$ 0.15 (0.50–0.80)	0.57 $\pm$ 0.09 (0.50–0.63)
ED	0.82 $\pm$ 0.04 (0.76–0.88)	0.84	0.91 $\pm$ 0.06 (0.84–0.97)	0.92 $\pm$ 0.00 (0.92–0.92)
SS	3.77 $\pm$ 0.23 (3.36–4.03)	4.20	4.17 $\pm$ 0.27 (3.86–4.37)	4.09 $\pm$ 0.06 (4.03–4.12)
VTL	1.39 $\pm$ 0.13 (1.23–1.60)	1.55	1.50 $\pm$ 0.19 (1.36–1.71)	1.50 $\pm$ 0.05 (1.46–1.53)
STL	0.55 $\pm$ 0.07 (0.46–0.67)	0.42	0.55 $\pm$ 0.04 (0.50–0.59)	0.67 $\pm$ 0.06 (0.63–0.71)
ODW	1.67 $\pm$ 0.18 (1.44–1.93)	1.53	1.46 $\pm$ 0.14 (1.33–1.61)	1.76 $\pm$ 0.23 (1.60–1.92)
PL	0.68 $\pm$ 0.06 (0.59–0.74)	0.81	0.67 $\pm$ 0.13 (0.52–0.76)	0.85 $\pm$ 0.06 (0.81–0.89)
AL	0.48 $\pm$ 0.03 (0.42–0.50)	0.50	0.62 $\pm$ 0.03 (0.59–0.64)	0.59 $\pm$ 0.07 (0.54–0.64)
GAP	0.47 $\pm$ 0.05 (0.40–0.54)	0.62	0.55 $\pm$ 0.04 (0.50–0.59)	0.57 $\pm$ 0.05 (0.54–0.60)
A1	1.16 $\pm$ 0.05 (1.08–1.21)	1.14	1.13 $\pm$ 0.07 (1.08–1.21)	1.23 $\pm$ 0.06 (1.19–1.28)
A2	1.07 $\pm$ 0.04 (1.01–1.11)	1.02	1.05 $\pm$ 0.04 (1.01–1.09)	1.18 $\pm$ 0.01 (1.18–1.19)
P1	1.02 $\pm$ 0.11 (0.82–1.11)	0.99	1.01 $\pm$ 0.10 (0.91–1.11)	1.19 $\pm$ 0.00 (1.19–1.19)
P2	0.98 $\pm$ 0.08 (0.82–1.08)	0.97	0.99 $\pm$ 0.04 (0.94–1.02)	1.18 $\pm$ 0.00 (1.18–1.18)
P3	0.67 $\pm$ 0.14 (0.50–0.84)	0.49	0.49 $\pm$ 0.04 (0.47–0.54)	0.76 $\pm$ 0.04 (0.74–0.79)
UJW	0.67 $\pm$ 0.00 (0.67–0.67)	0.67	0.56 $\pm$ 0.10 (0.50–0.67)	0.67 $\pm$ 0.00 (0.67–0.67)
UJL	0.74 $\pm$ 0.05 (0.67–0.82)	0.94	0.81 $\pm$ 0.04 (0.76–0.84)	0.77 $\pm$ 0.07 (0.77–0.77)

Allobates sp. nov. clado *trilineatus*



CAPÍTULO 3

Jesus R.D. Souza, Miquéias Ferrão, Igor Luis Kaefer, James Hanken e Albertina P. Lima. Taxonomia integrativa revela uma terceira espécie de *Allobates* do clado *trilineatus* (Anura: Aromobatidae) do alto rio Purus, sudoeste da Amazônia. Manuscrito em preparação para o periódico Vertebrate Zoology.

Taxonomia integrativa revela uma terceira espécie de *Allobates* do clado *trilineatus* (Anura: Aromobatidae) do alto rio Purus, sudoeste da Amazônia

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Abstract

We used integrative taxonomy to describe a new species of nurse frog of the genus *Allobates* for the southwestern Brazilian Amazonia. The new species belongs to the *Allobates trilineatus* complex, with a genetic distance for 16S samples of 2.6% between the new species and *Allobates trilineatus* sensu stricto. The new species differs from its congeners mainly by the gray to bluish-gray throat in live males; fingers II and III swollen; advertisement calls composed by two notes, emitted solitarily or in series of two to seven calls, with a dominant frequency of 4,996-5,965 Hz; exotrophic tadpoles with six short pyramidal papillae on the anterior labium and nine to eighteen short cylindrical papillae on the posterior labium.

Key words: advertisement call, morphology, tadpole, phylogeny, state of Acre

Introdução

O gênero neotropical de rãzinhas cuidadoras *Allobates* Zimmermann & Zimmermann, 1988 é notoriamente conhecido por apresentar morfologia conservada (Kaefer et al., 2013; Fernandes et al., 2021) e abrigar complexos de espécies crípticas (e.g., Lima et al., 2020; Ferrão et al., 2022, Souza et al., in press). Por esse motivo e pela necessidade de diagnoses robustas, geralmente baseadas em caracteres morfológicos e bioacústicos para diferenciar as linhagens, boa parte das espécies descritas anteriormente ao amplo uso da taxonomia integrativa apresentam inconsistências taxonômicas. Um exemplo emblemático de tal problemática é o de *Allobates trilineatus* (Boulenger, 1884). A espécie foi descrita com base em apenas um exemplar coletado por Hahnel, em Yurimaguas, rio Hualaga, norte do Peru. A descrição é sucinta, seguindo os padrões da época. Dentre os caracteres observados, “a presença de uma linha vertebral estreita, com coloração igual às linhas dorsolaterais”, provavelmente inspirou o epíteto específico. O uso deliberado do nome, o qual foi atribuído a populações de *Allobates* em diferentes localidades, ao longo de uma considerável porção do bioma amazônico apontava que o táxon se tratava de complexo de espécies crípticas, especialmente em virtude da grande variação intraespecífica observada desde sua descrição original (Jaramillo et al, 2021).

A primeira análise detalhada da espécie foi realizada por Grant & Rodríguez (2001) que examinaram cinco espécimes da localidade tipo (incluindo o holótipo) e 118 outros espécimes oriundos de 13 localidades do Equador e Peru. Estes autores forneceram uma redescrição composta que incluiu a descrição de cantos de anúncio a partir de três dessas localidades

(Yurimaguas, Panguana e Cuzco Amazônico). Posteriormente, Grant et al. (2006), produziram os primeiros dados moleculares atribuídos à *Allobates trilineatus*, oriundos de Puerto Maldonado e Panguana no Peru. Essas amostras, bem como outras produzidas a partir do Peru e Brasil (Parque Nacional da Serra do Divisor, Porto Walter e Rio Branco, no Acre) e atribuídas a *Allobates trilineatus*, foram incluídas em subsequentes estudos filogenéticos (Santos et al., 2009; Grant et al., 2017; Melo-Sampaio et al., 2018, 2020). A grande variação intraespecífica, incluindo caracteres morfológicos e acústicos, e o fato dessas amostras distarem mais de 400 km da localidade tipo de *Allobates trilineatus*, claramente indicavam a necessidade de aquisição de material topotípico para a confirmação da identidade e distribuição da espécie (Grant et al., 2017; Melo-Sampaio et al., 2018).

Recentemente, Jaramillo et al. (2021) redescreveram *Allobates trilineatus* com base em material coletado (exemplares e DNA) obtido a partir de Vila Munichs, distrito de Yurimaguas, localidade tipo da espécie. Os autores geraram uma filogenia completa do clado, forneceram uma definição corrigida da espécie e resolveram inconsistências morfológicas e bioacústicas entre exemplares atribuídos à espécie que haviam sido examinados por Grant & Rodriguez (2001). Essas informações possibilitaram ainda a descrição de uma nova espécie-irmã, *Allobates sieggreenae* (Gagliardi-Urrutia et al., 2021) que ocorre a cerca de 280 km da localidade-tipo de *Allobates trilineatus*, clarificando assim, diversos questionamentos acerca da alfa taxonomia desta espécie. Jaramillo et al. (2021) sugerem ainda que com base em evidência molecular (< 3% de distância genética), os espécimes do estado brasileiro do Acre e do Peru (Panguana Huanuco e Puerto Maldonado), classificados como *Allobates* aff. *trilineatus*, carecem de exame morfológico e bioacústico.

Apesar dos avanços significativos, *Allobates trilineatus* ainda representa um complexo de espécies amplamente distribuído e, portanto, é necessário acesso às áreas de distribuição e ocorrência das linhagens que compõem o complexo. Novas amostragens possibilitam acesso a diferentes classes de caracteres e, conseqüentemente, permitem o reconhecimento formal de novos táxons com base em múltiplas linhas de evidência.

Neste contexto, utilizando dados, morfológicos de adultos e girinos, em conjunto com informações bioacústicos e moleculares descrevemos uma nova espécie de *Allobates* pertencente ao complexo *Allobates trilineatus* que ocorre na região sudoeste da Amazônia, no estado brasileiro do Acre. A área situa-se no município de Manuel Urbano, a cerca de 850 km de distância em linha reta da localidade-tipo de *Allobates trilineatus* sensu stricto. O acesso à área é realizado pela rodovia BR 364 ou através do curso do rio Purus. A espécie foi registrada

em ambas as margens do rio Purus e sua localidade-tipo é um remanescente florestal com pequenos riachos e árvores que não ultrapassam 20m de altura, permeado por pequenos igarapés e poças temporárias que compõem a reserva legal de uma propriedade particular. O novo táxon ocorre em simpatria com *Allobates albiventris* (Souza et al. in press), mas a amplitude de sua distribuição geográfica permanece desconhecida, tendo em vista que não foi possível percorrer grandes distancias o longo meândrico do rio Purus.

Material e métodos

Amostragem

Espécimes adultos, girinos e desovas foram coletados em janeiro de 2019, janeiro e fevereiro de 2020, na margem da rodovia BR 364 (8°52'27.43" S, 69°17'7.76" O, 166m de altitude), município de Manuel Urbano, Acre, Brasil. Desovas foram coletadas após observação de acasalamentos e girinos foram criados em laboratório até atingirem estágios ontogenéticos adequados para caracterizações morfológicas. Fotografias foram obtidas em campo e em laboratório para permitir a descrição de características fenotípicas tais como morfologia, coloração e comportamento.

Adultos foram mortos com solução de Benzocaína 2%, preservados em formaldeído 10% e armazenados em álcool 70%. Girinos foram mortos com solução de Benzocaína 2% diluída em água e preservados em formaldeído 10%. Amostras de tecido muscular foram retiradas antes da preservação dos espécimes em formol e foram estocadas em álcool 95% para análises genéticas. Adultos e girinos foram depositados na Coleção Herpetológica de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia (*vouchers* INPA-H XXXX-XXXX).

Cantos de anúncio foram gravados por JRDS e APL, a cerca de 1 m do animal, com gravadores digitais Sony D50 e Zoom H4n acoplados a um microfone direcional (sample rate = 44.1 kHz; sample size = 16 bits). A temperatura do ar foi obtida através de termômetro digital no local da gravação de cada macho gravado. Gravações foram armazenadas em arquivos não comprimidos (formato WAV) e depositadas na Fonoteca Neotropical Jacques Viellard (FNJV), Unicamp, Campinas, Brasil. *Voucher* de gravações estarão disponíveis no repositório digital SAPOTECA do INPA, Manaus, Brasil (ppbio.inpa.gov.br/sapoteca/paginainicial).

Sequenciamento e análise filogenética

DNA genômico foi extraído a partir de amostras de tecido muscular de oito indivíduos da nova espécie de *Allobates* de duas localidades no estado do Acre (Brasil): Manoel Urbano (n = 6) e

Três Bocas (n = 2). As extrações de DNA foram realizadas utilizando o kit Wizard (Promega Corp., Madison, WI, EUA) de acordo com as recomendações do fabricante. Fragmentos do gene mitocondrial 16S rRNA foram amplificados por meio de Reações de Cadeia Polimerase (PCR) utilizando os *primers* universais 16sar (CGCCTGTTTATCAAAAACAT) e 16sbr (CCGGTCTGAACTCAGATCACGT) descritos por Palumbi (1996). A concentração de reagentes e a ciclagem utilizadas na PCR, bem como a purificação dos produtos resultantes, seguiram aquelas descritas em Souza et al. (in press). Os produtos purificados de RCP foram sequenciados no sequenciador automático ABI Prism 3130 (ThermoFisher Scientific, Waltham, EUA). As sequências resultantes foram checadas visualmente e editadas no GENEIOUS 5.3.4 (Kearse et al. 2012), sendo posteriormente depositadas no GenBank (www.ncbi.nlm.nih.gov/Genbank). A obtenção dos dados moleculares foi realizada no Laboratório Temático de Biologia Molecular (LTBM) do Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brasil.

Sequências de 16S do complexo *Allobates trilineatus* previamente depositadas no GenBank e com alto grau de similaridade com as da nova espécie foram identificadas por meio da ferramenta BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) e incorporadas ao banco de dados. Além destas, sequências 16S representativas das demais espécies nominais e candidatas de *Allobates* disponíveis no GenBank foram obtidas. Para trazer maior cobertura gênica ao banco de dados, foram acessadas sequências dos genes 12S rRNA, citocromo c oxidase I (COI), citocromo b (CYTB) e NADH desidrogenase 1 (ND1). Sequências de *Ameerega* Bauer (1986), *Anomaloglossus* Grant et al. (2006), *Aromobates* Myers, Paolillo-O. e Daly (1991), *Colostethus* Cope (1866), *Dendrobates* Wagler (1830), *Leucostethus* Grant et al. (2017), *Mannophryne* La Marca (1992), *Phyllobates* Bibron (1840), *Rheobates* Grant et al. (2006) e *Silverstoneia* Grant et al. (2006) foram utilizadas para representar grupos externos. A amostragem de táxon seguiu principalmente Melo-Sampaio, Oliveira & Prates (2018), Réjaud et al. (2020) e Vacher et al. (2020), enquanto a amostragem gênica seguiu Réjaud et al. (2020).

As sequências foram alinhadas independentemente para cada gene através da implementação web de MAFFT (<https://mafft.cbrc.jp/>) utilizando parâmetros padrões das estratégias E-INS-i e G-INS-i para genes não codificadores e codificadores de proteínas (Katoh & Standley 2013), respectivamente. Posteriormente, os alinhamentos foram concatenados com Mesquite 3.6 (Maddison & Maddison, 2019) para formar a matriz final de sequências composta por 6.255 pares de bases e 123 terminais. ModelFinder (Kalyaanamoorthy et al. 2017) foi utilizado para avaliar o melhor esquema de partição e seus respectivos modelos de evolução

nucleotídica. Foram avaliadas inicialmente 10 partições, sendo uma para os genes não codificadores de proteína e uma para cada códon dos genes codificadores, o que resultou nos seguintes modelos e esquemas: TIM2+F+R5 para 12S+16S; TIM3e+I+G4 para 1º códon do COI; TIM+F+I+G4 para 2º códon do COI, CYTB e ND1; TN+F+R5 para o 3º códon do COI, CYTB e ND1; e SYM+I+G4 para o 1º códon do CYTB e ND1.

As relações filogenéticas foram inferidas por meio de máxima verossimilhança (ML) em IQ-TREE (Trifinopoulos et al. 2016). O suporte das relações foi calculado por meio de 10.000 réplicas de aproximação de *bootstrap* ultrarrápidas (Hoang et al. 2018) com 10.000 iterações máximas, coeficiente de correlação mínimo de 0,99 e 10.000 réplicas do teste de Shimodaira-Hasegawa. Distâncias genéticas não corrigidas (p) e Kimura-2-parâmetros (K2P; Kimura 1980) para o gene 16S foram calculadas entre a linhagem da nova espécie e as demais linhagens do complexo *Allobates trilineatus* utilizando-se MEGA 6 (Tamura et al. 2013). As extremidades do alinhamento foram cortadas para evitar erros associados o excesso de dados faltantes nessas regiões, resultando em um alinhamento final com 574 pares de bases.

Caracteres morfológicos

O sexo dos indivíduos foi determinado em campo através da observação direta de machos vocalizando, pela coloração da região ventral (machos têm papo escuro e fêmeas têm região ventral amarela) ou pelo comportamento durante interações reprodutivas. Medidas morfométricas de adultos foram tomadas com uso de paquímetro digital e estereomicroscópio com lente graduada (precisão de 0,1mm) seguindo Barrio-Amorós & Santos (2009) e Lima et al. (2007). Terminologias e caracteres seguem Grant et al. (2006) e o grau de inchaço dos dedos da mão segue Cavalcanti et al. (2021). As medidas lineares tomadas em adultos foram: comprimento rostro-cloacal (**SVL**); comprimento da cabeça, da articulação da maxila até a ponta do focinho (**HL**); distância interorbital, medida entre os limites internos das pálpebras superiores (**IO**); largura da cabeça ao nível da articulação da maxila (**HW**); comprimento do focinho, do canto anterior do olho até a ponta do focinho (**SL**); distância do olho à narina, do canto anterior do olho ao centro da narina (**END**); distância entre narinas, medido entre o limite interno entre as narinas (**IN**); comprimento do olho, do canto anterior ao posterior (**EL**); diâmetro do tímpano, medido horizontalmente no eixo crânio-cauda (**TYM**); comprimento do antebraço, da extremidade proximal do tubérculo palmar até a borda exterior do cotovelo flexionado (**FAL**); comprimento do braço, da sua inserção no tronco até a borda exterior do cotovelo flexionado (**UAL**); comprimento do fêmur (coxa) à partir do centro da abertura anal

até a articulação tíbio-femoral, com os membros semiflexionados (**LL**); comprimento da tíbia, da borda externa do joelho flexionado até o calcanhar (**TL**); comprimento do pé, da extremidade proximal do tubérculo metatarsal até a ponta do dedo IV (**FL**); Comprimento da mão da borda proximal do tubérculo palmar até a ponta do dedo I- IV (**HANDI-IV**); largura do disco no dedo III da mão (**WFD**); diâmetro do tubérculo palmar (**DPT**); largura do tubérculo tenar (**WTT**); largura do dedo III da mão (**WPF**); largura do disco no dedo IV do pé (**WTD**).

Os estágios de desenvolvimento de girinos foram determinados de acordo com Gosner (1960). Medidas morfométricas foram tomadas através de estereomicroscópio com lente graduada (precisão de 0,1mm). Terminologias, caracteres diagnósticos e medidas morfométricas seguiram Altig & McDiarmid (1999) e Schulze et al. (2015). Caracterização do corpo segue Dubeux et al. (2020). As medidas tomadas foram: comprimento total (**TL**), medido da ponta da cauda até a ponta de focinho; comprimento do corpo (**BL**), medido da ponta do focinho à inserção da cauda; comprimento da cauda(**TAL**), medido da ponta da cauda até sua inserção no corpo; largura do corpo no nível do espiráculo (**BW**); altura do corpo no nível do espiráculo (**BH**); largura da cabeça no nível dos olhos (**HWLE**); largura máxima do músculo da cauda (**TMW**); altura máxima da cauda (**MTH**); altura máxima do músculo da cauda (**TMH**); distância interorbital (**IOD**); distância entre narinas (**IND**); distância olho–narina (**END**); distância narina–focinho (**NSD**); diâmetro do olho (**ED**); comprimento do tubo anal (**VTL**); comprimento do espiráculo (**STL**); largura do disco oral (**ODW**).

Descrição das vocalizações

Para definição das terminologias e classificação das vocalizações, utilizamos a abordagem centrada em notas, conforme Köhler et al. (2017). Foram medidos os seguintes parâmetros espectrais (em Hz) e temporais (em ms) das vocalizações de anúncio: duração das notas (**ND1** e **ND2**) de cada canto; intervalo entre notas (**NI**); duração do canto (**CD**), medindo-se as duas notas mais o intervalo; intervalo entre cantos (**CI**); duração das séries de cantos (**CSD**); intervalo entre séries de cantos (**ICSI**); frequência mais baixa da primeira e segunda nota (**LFN1**, **LFN2**); frequência mais alta da primeira e segunda nota (**HFN1**, **HFN2**); frequência dominante da primeira e segunda nota (**DFN1**, **DFN2**) e taxa de repetição (**RR**), número de cantos por segundo. Medidas espectrais foram realizadas no espectro de energia (Window = Blackman; Discrete Fourier Transform = 2048 samples; 3dB filter bandwidth = 80.0 Hz). Mensuração dos parâmetros acústicos foi realizada em Raven 1.5 (Bioacoustics Research

Program, 2015) e figuras foram geradas em ambiente computacional R através do pacote Seewave (Seuer et al., 2008).

Comparações interespecíficas

A nova espécie é comparada às espécies de *Allobates* crpticamente coloridos, distribuídas ao sul do rio Solimões-Amazonas, sudoeste da Amazônia e parte da Amazônia central: *A. bacurau* Simões, 2016; *A. caeruleodactylus* (Lima & Caldwell, 2001); *A. caldwella* Lima, Ferrão, & Silva, 2020; *A. conspicuus* (Morales, 2002); *A. flaviventris* Melo-Sampaio, Souza, & Peloso, 2013; *A. fuscillus* (Morales, 2002); *A. gasconi* (Morales, 2002); *A. grillisimilis* Simões et al., 2013; *A. grillicantus* Moraes & Lima, 2021; *A. kamilae* Ferrão, Hanken, & Lima, 2022; *A. melanolaemus* (Grant & Rodríguez, 2001); *A. nidicola* (J. P. Caldwell & Lima, 2003); *A. ornatus* (Morales, 2002); *A. pacaas* Melo-sampaio et al., 2020; *A. paleci* Silva et al., 2022; *A. paleovarzensis* Lima et al., 2010; *A. sieggreenae* Gagliardi-Urrutia et al., 2021; *A. subfolionidificans* (Lima, Sanchez & Souza, 2007); *A. tinae* Melo-Sampaio, Oliveira & Prates, 2018; *A. trilineatus* Jaramillo et al. 2021; *A. vanzolinius* (Morales, 2002); e *A. velocicantus* Souza et al., 2020 (Fig. 1).

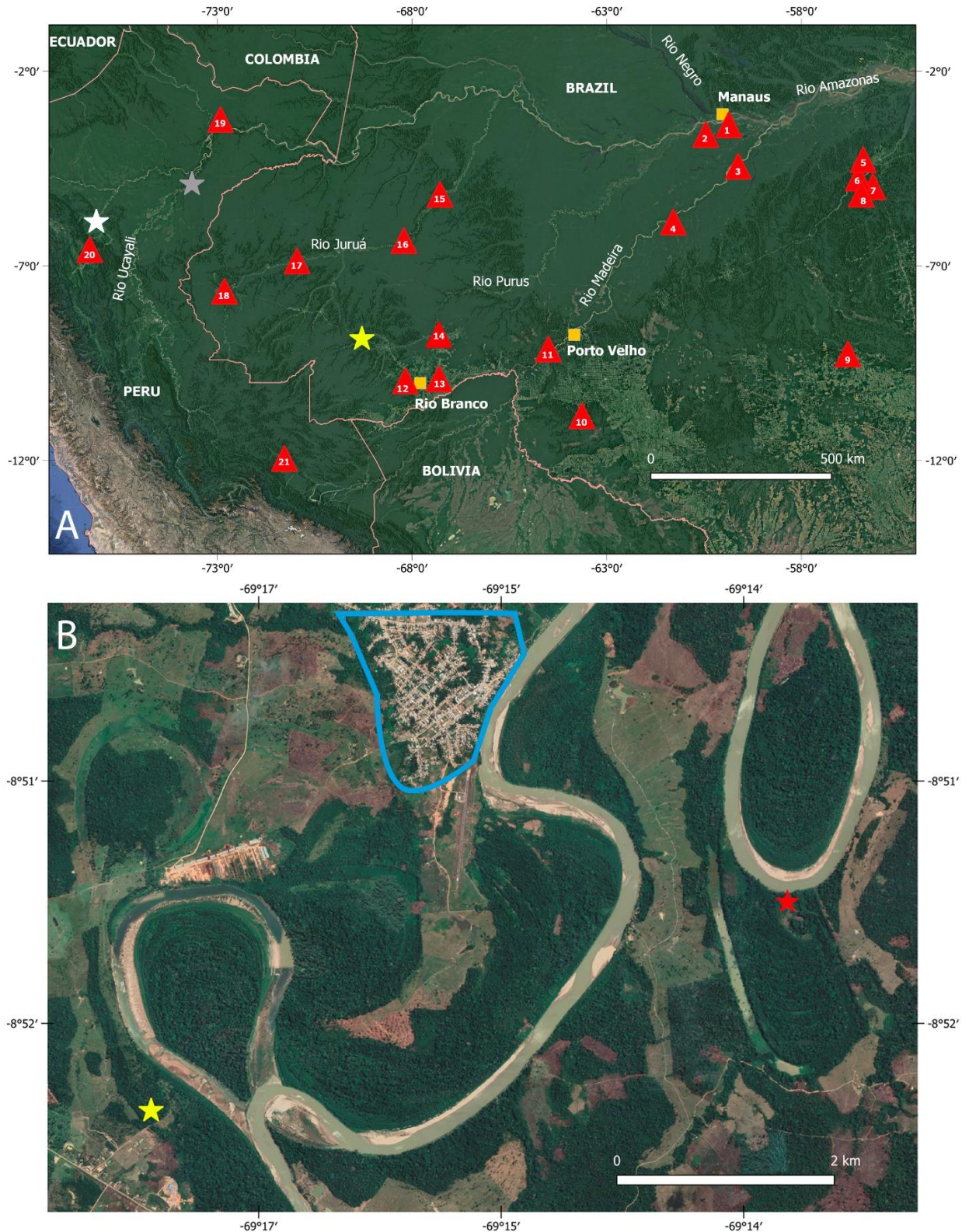


Figura 1.(A) Distribuição de *Allobates* sp. nov. (estrela amarela), em relação a *Allobates trilineatus* sensu stricto (estrela branca), *Allobates sieggreenae* sensu stricto (estrela cinza) e a outros *Allobates* descritos para a região (triângulos vermelhos: 1- *caldwellae* e *paleovarzensis*; 2-*caeruleodactylus* e *nidicola*; 3-*grillissimilis*; 4-*bacurau*; 5-*tapajos*; 6-*magnussoni*; 7-*grillicantus*; 8-*nunciatus*; 9-*paleci*; 10-*pacaas*; 11-*kamilae*; 12-

subfolionidificans; 13-*flaviventris*; 14-*tinae*; 15-*vanzolinius*; 16-*gasconi*; 17-*fuscus*; 18-*velocicantus*; 19-*melanolaemus*; 20- *ornatus* 21- *conspicuus*). Quadrados laranja são as capitais do Acre (Rio Branco), Rondônia (Porto Velho) e Amazonas (Manaus). (B) Vista aérea de um trecho do rio Purus, mostrando a localidade-tipo da espécie (estrela amarela), o local de coleta dos parátipos (estrela vermelha) e o município de Manoel Urbano (área circundada em azul).

Resultados

Relações filogenéticas

A monofilia de *Allobates* é recuperada na filogenia molecular e é consistente com o mais recente estudo filogenético sobre o grupo (Réjaud et al., 2020) (Fig. 2). O clado *Allobates olfersioides* é recuperado como irmão de todas as demais espécies do grupo, assim como os principais clados reconhecidos atualmente (sensu Réjaud et al., 2020) são bem suportados. As relações filogenéticas entre as espécies são similares àquelas encontrada por Réjaud et al. (2020), com exceção das que envolvem os clados *A. kingsburyi* e *A. femoralis*. *Allobates kingsburyi* agrupou-se com o grande clado que reúne as espécies pertencentes aos clados *A. goianus*, *A. caeruleodactylus* e *A. trilineatus*, enquanto *A. femoralis* agrupa-se com todos estes clados juntos.

A filogenia recuperou o complexo *Allobates trilineatus* formado por *Allobates sieggreenae* sensu stricto, *Allobates trilineatus* sensu stricto, quatro espécies candidatas nomeadas *Allobates* aff. *trilineatus* clados 1-4 (CL1-4), *Allobates fuscus* sensu stricto e a nova espécie que denominamos *Allobates* **sp. nov.** (Fig. 2). *Allobates sieggreenae* sensu stricto é fortemente suportado como clado irmão a todos os demais clados que compõem o complexo *Allobates trilineatus*. *Allobates trilineatus* sensu stricto é irmão de *Allobates* aff. *trilineatus* CL2. A nova espécie se agrupa com os clados *Allobates* aff. *trilineatus* CL3 e CL4, com forte suporte, formando um grupo irmão do clado *Allobates fuscus* sensu stricto, com indivíduos de várias localidades no Amazonas e no Acre (Brasil) (Fig. 2). *Allobates sieggreenae* sensu stricto apresentou a maior distância genética com a nova espécie (5.1%), enquanto CL3 e CL4, as menores (1.1 e 1.0 %, respectivamente). A distância genética entre a nova espécie e *Allobates fuscus* sensu stricto e *Allobates trilineatus* sensu stricto é de 2% e 2.6%, respectivamente (Tabela 1).



Figura 2. Filogenia de *Allobates* baseada em cinco genes mitocondriais (12S, 16S, COI, Cytb e ND1), focando nas relações dentro do complexo *Allobates trilineatus* (região cinza, em destaque).

Tabela 1. Distâncias genéticas (%) *p* (diagonal superior) e K2P (diagonal inferior) entre linhagens do complexo *Allobates trilineatus*. Valores em negrito correspondem à distância *p* entre indivíduos de uma mesma linhagem. Abreviações: SS, sensu stricto.

Linhagens	1	2	3	4	5	6	7	8
1 <i>A. sieggreenae</i> SS	0.5	5.0	5.1	5.0	4.8	5.1	4.9	4.9
2 <i>A. fuscillus</i> SS	5.2	1.1	2.6	2.1	2.1	2.3	2.0	2.0
3 <i>A. trilineatus</i> SS	5.4	2.7	0.5	2.9	2.7	2.9	2.8	2.5
4 <i>A. aff. trilineatus</i> Clado 1	5.2	2.1	3.0	0.1	2.0	2.3	1.9	2.2
5 <i>A. aff. trilineatus</i> Clado 2	4.9	2.2	2.8	2.0	0.0	2.2	2.2	1.9
6 <i>A. aff. trilineatus</i> Clado 3	5.3	2.3	3.0	2.3	2.2	0.0	1.3	1.1
7 <i>A. aff. trilineatus</i> Clado 4	5.1	2.0	2.8	2.0	2.3	1.3	0.7	1.0
8 <i>Allobates</i> sp. nov.	5.1	2.0	2.6	2.2	1.9	1.1	1.0	0.1

Taxonomia

¹*Allobates* sp. nov.

Holótipo. INPA-H XXXX (número de campo APL21378), macho adulto coletado na margem da BR 364, município de Manuel Urbano, estado do Acre (8°52'27.43" S, 69°17'7.76" O, 166 m de altitude), em 11 de janeiro de 2019 por JRDS e APL.

Paratopótipos. Dezesseis espécimes na mesma localidade do holótipo: seis machos INPA-HXXXXXX–XX (números de campo APL21376–77, 21379, 21482–83, 21486, respectivamente) coletados em 11, 24–25 de janeiro de 2019 por JRDS e APL; um macho INPA-H (número de campo APL21510) coletado em 7 de janeiro de 2020 por JRDS; um macho INPA-H XXXXX (número de campo APL21529) coletado em 5 de fevereiro de 2020 por JRDS; quatro fêmeas INPA-H XXXXX–XX (números de campo APL21475, 21477, 21484–85, respectivamente) coletadas em 24 de janeiro de 2020 por JRDS e APL; três fêmeas INPA-HXXXXXX–XX (números de campo APL21513–14, 21516, respectivamente) coletadas em 13 de janeiro de 2020 por JRDS e uma fêmea INPA-H (número de campo APL21519) coletada em 18 de janeiro de 2020 por JRDS.

¹ O epíteto específico foi propositalmente omitido, por conta do código de nomenclatura zoológica.

Parátipos. Três espécimes machos, INPA-HXXXX-XX (números de campo APL21490-92), coletados por APL e JRDS em 25 de janeiro de 2019, na localidade Três Bocas, margem direta do rio Purus (8°51'19" S, 69°13'58" O, 169 m de altitude).

Etimologia. O epíteto específico é uma homenagem ao escritor brasileiro Euclides da Cunha, que após escrever e alcançar fama com o primeiro romance-reportagem brasileiro "Os Sertões", desejava conhecer e descrever a Amazônia. Em 1904 chefiou a comissão mista de brasileiros e peruanos para reconhecimento das nascentes do rio Purus, que serviria de base para traçar os limites oficiais entre Peru e o território do Acre, terra renegada pelo governo brasileiro, cujos habitantes lutaram com armas para pertencer ao Brasil. Euclides da Cunha foi o primeiro cientista brasileiro a conhecer de fato a Amazônia, e a fazer uma profunda leitura da “sociedade da borracha”, denunciando as condições miseráveis e escravidão em que cidadãos nordestinos eram submetidos para a extração do látex.

Posicionamento genérico

A nova espécie é alocada ao gênero *Allobates* com base em evidência molecular apresentada neste estudo (Fig. 2), nas similaridades gerais na morfologia externa com outros congêneres e pela seguinte combinação de caracteres (sensu Grant et al. 2006): ponta do dedo IV não alcança o tubérculo subarticular distal do dedo III (*character* 4); dedo III inchado em machos adultos (*character* 20); presença de marcas paracloacais claras (*character* 49); presença de linha dorsolateral clara (*character* 52); presença de linha ventrolateral clara (*character* 54) e ausência de excrescência nupcial no dedo II de machos (*character* 24) e fêmeas (*character* 26).

Definição

Allobates **sp. nov.** caracteriza-se pela seguinte combinação de estados caracteres: (1) pequeno tamanho, com SVL de 15.3–16.4 mm em machos e 15.6–16.5 mm em fêmeas; (2) textura da superfície dorsal lisa, tubérculos pouco evidentes, esparsos aleatoriamente; (3) focinho semitruncado e levemente arredondado em vista dorsal; (4) *Canthus rostralis* ligeiramente côncavo, com região loreal plana; (5) narinas visíveis lateral e ventralmente; (6) dentição presente, vista sob magnificação; (7) tímpano corresponde a 35,6–44,2% do diâmetro do olho; (8) processo lingual médio ausente; (9) escudos dorsais digitais pareados presentes; (10) cume metacarpal presente; (11) tubérculos supernumerários ausentes; (12) excrescências nupciais no polegar ausentes; (13) dedos II-III da mão inchados em machos; forte inchaço do dedo III, da

base do dedo ao disco; (14) dedo IV da mão não alcança o tubérculo distal do dedo III, quando justapostos; (15) quilhas laterais dos dedos das mãos presentes; no dedo I estende-se até a borda anterior do tubérculo subarticular basal; (16) discos dos dedos da mão moderadamente expandidos; disco do dedo III em machos igual ou levemente maior que a falange; (17) dedo IV apenas com tubérculo subarticular basal; (18) linha dorsolateral clara presente, larga e bem visível, de coloração creme, com melanóforos distribuídos uniformemente; (19) Linha vertebral (presente em 60% dos espécimes) de coloração igual à linha dorsolateral clara, estendendo-se do focinho à região próxima ao uróstilo; (20) faixa lateral marrom ao redor do corpo, estreita no focinho, gradativamente mais larga, até a região inguinal; bordas superior e inferior regulares; (21) linha ventrolateral clara presente, bem visível, completa, distribuindo-se do focinho à virilha; em alguns espécimes inicia na região logo abaixo do olho; pode ter um interrupção pequena na região mediana do corpo; (22) linha obliquo lateral presente, pouco visível; difusa em espécimes vivos; (23) quilha tarsal presente, tubercular, em forma de “vírgula” invertida; (24) franja tarsal ausente; (25) franja metatarsal presente, tubercular na região proximal, achatada e filamentosa distalmente, em direção ao dedo V do pé; (26) quilhas laterais presentes em todos os dedos dos pés; (27) discos dos dedos II-V dos pés moderadamente expandidos; disco do dedo I do pé com largura igual à falange distal; (28) membrana basal presentes nos dedos II-IV dos pés; rudimentar entre os dedos II-III; (29) marcas paracloacais presentes, beje, com ou sem melanóforos, em forma de “c”; (30) superfície dorsal dos braços, antebraços e mãos creme, com melanóforos marrom escuro espalhados uniformemente; manchas marrom escuro de tamanhos variados presentes em alguns indivíduos; (31) superfície dorsal da coxa e tíbia beje, com melanóforos espalhados uniformemente; manchas marrom escuros de tamanhos presentes em alguns indivíduos; (32) barra transversal presente somente na tíbia; (33) superfície dorsal do tarso e pés creme com melanóforos; manchas marrom escuro de tamanhos variados podem estar presentes; (34) coloração de fundo da superfície dorsal do corpo creme, com melanóforos; uma faixa retangular marrom, limitada pelas linhas dorsolaterais claras estende-se da região anterior aos olhos até o uróstilo; (35) em machos, garganta cinza escuro e peito cinza claro, ambos com alta concentração de melanóforos; barriga creme, com melanóforos distribuídos uniformemente; ; (36) em fêmeas, garganta e peito creme, sem melanóforos ou em pequena concentração, distribuídos esparsamente na região gular, ou concentrados na região anterior da mandíbula; barriga creme sem melanóforos ou em baixa concentração distribuídos uniformemente; (37) língua creme, mais comprida que larga, redonda na porção posterior, com porção anterior atachada ao chão da boca; (38) testículos não

pigmentado; (39) ovócitos maduros de coloração castanho claro; (40) intestino grosso não pigmentado; (41) canto de anúncio composto por duas notas, emitido solitariamente ou em séries de 2 a 7 cantos, com frequências dominantes que variam de 4.996-5.965 Hz.

Cor em vida. Superfície dorsal marrom chocolate, com grânulos marrom escuro de tamanhos variados, distribuídos ao longo do dorso; linha dorsolateral clara marrom claro; linha vertebral, da mesma cor que a linha dorsolateral; linha ventrolateral clara branca em machos, branco-amarelada em fêmeas; faixa lateral ao redor do corpo de coloração marrom café; linha oblíquo-lateral clara formada por diminutos pontos de cor cinza, dentro da faixa lateral marrom, estendendo-se da região inguinal à região mediana do corpo; superfície dorsal dos braços, antebraços e mãos marrom-alaranjado translúcido; superfície dorsal da coxa e tíbia marrom claro a cinza, com manchas marrom escuro de tamanhos variados; barra transversal na tíbia marrom escuro; marca paracloacal bronze alaranjada; **em machos**, garganta e peito cinza escuro a cinza violáceo. Saco vocal simples subgular, quando inflado de coloração cinza a cinza azuláceo. Manchas marrom escuro fosco e brancas podem ser observadas nas regiões marginais do saco vocal; barriga creme amarelada a cinza. Manchas brancas presentes na região ventrolateral logo abaixo da linha ventrolateral clara; região ventrolateral do corpo cinza a cinza translúcido. Manchas brancas de tamanhos variados podem estar presentes; **em fêmeas**, garganta, peito e barriga amarelo translúcido em fundo branco; região ventrolateral do corpo creme a amarelo translúcido, manchas brancas de tamanhos variados podem estar presentes. Íris bronze metálico; pupila negra, redonda (Figs. 3 e 4).



Figura 3. Padrão de coloração dorsal e ventrolateral em machos e fêmeas vivos de *Allobates* sp. nov. (A, B e D, machos não coletados; C, macho APL21510; E, fêmea APL21516 e F, fêmea APL21514). Note: a linha vertebral, de coloração igual às linhas dorsolaterais, presente em 60% dos espécimes (B, F); a linha oblíquo-lateral, de coloração cinza, dentro da faixa lateral marrom (todas exceto, B); a estreita marca paracloacal em forma de “c”, com coloração bronze alaranjada (A, D) e a coloração cinza a cinza azulado do saco vocal inflado (A, B). Fotografias de JRDS.



Figura 4. Padrão de coloração ventral em machos e fêmeas de *Allobates* **sp. nov.** vivos. (A) fêmea APL21519; (B) fêmea APL21381; (C) fêmea APL21516; (D) macho APL 21377, (E) macho APL21376 e (F) macho APL21379. Fotografias de JRDS e APL.

Diagnose

Caracteres diagnósticos das espécies comparadas à espécie nova são apresentados entre parênteses, salvo se explicitamente ao contrário. *Allobates* **sp. nov.** é facilmente distinto das espécies de *Allobates* brilhantemente coloridas (*A. femoralis*, *A. hodli*, *A. zaparo* e *A. myersi*) pela ausência de marcas amarelo, laranja ou vermelho-brilhante nas coxas, e pela ausência de textura granular ou coloração vermelha do dorso (ao menos uma característica presente nestas espécies).

Machos de *Allobates* **sp. nov.** possuem SVL de 15.3 a 16.4 mm, distinto de *A. bacurau* e *A. pacaas*, cujos machos destas espécies possuem SVL máximo < 15.3 mm [14.7 mm em *A.*

bacurau; 15.2 mm em *A. pacaas*] e de *A. flaviventris*, *A. melanolaemus*, *A. nidicola*, *A. nunciatus*, *A. paleovarzensis* e *A. vanzolinus*, os quais possuem SVL mínimo > 16.4 mm [16.7 mm em *A. flaviventris*; 21.1 mm em *A. melanolaemus*; 18.5 mm em *A. nidicola*; 19.2 mm em *A. nunciatus*; 18.3 mm em *A. paleovarzensis* e de 21.5 mm em *A. vanzolinus*]. Também é possível distingui-lo de *A. magnussoni* pela amplitude do SVL em machos, que varia de 16.1-19.6 mm.

Machos de *Allobates* **sp. nov.** possuem garganta cinza escuro a violáceo, o que o distingue de *A. caeruleodactylus*, *A. conspicuus*, *A. subfolionidificans*, *A. grillisimilis*, *A. grillicantus*, *A. kamilae*, *A. sieggreenae*, *A. caldwella*, *A. tinae*, *A. paleci*, *A. tapajos* e *A. velocicantus* [branca em *A. caeruleodactylus*, *A. conspicuus* e *A. subfolionidificans*; branca a creme translúcido em *A. grillisimilis* e *A. sieggreenae*; amarelo em *A. paleci*, *A. grillicantus* e *A. velocicantus*; amarelo ouro em *A. caldwella*, *A. tinae* e *A. tapajos*; amarelo brilhante em *A. kamilae*].

Allobates **sp. nov.** não possui marcas ou desenhos no dorso, o que o diferencia de *A. flaviventris*, *A. magnussoni*, *A. gasconi*, *A. ornatus*, *A. pacaas* e *A. paleci* (manchas escuras ou desenhos padrão romboides, forma de diamantes, ampolhetas ou triângulos no dorso destas espécies). Adicionalmente, *Allobates* **sp. nov.** pode se diferenciar de *A. caeruleodactylus* pelos escudos digitais branco em vida (azul); de *A. conspicuus*, *A. caldwella*, *A. flaviventris*, *A. grillisimilis*, *A. grillicantus*, *A. melanolaemus*, *A. nidicola*, *A. pacaas*, *A. paleovarzensis*, *A. subfolionidificans*, *A. tapajos* e *A. tinae* pelo forte inchaço no dedo III dos machos (fracamente inchado em *A. melanolaemus*, *A. grillicantus* e *A. paleovarzensis*; ausência de inchaço nas demais espécies); de *A. paleci*, *A. sieggreenae* e *A. velocicantus*, pela presença da linha dorsolateral clara (ausente em *A. paleci*; irregular e difusa em *A. sieggreenae*; incompleta em *A. velocicantus*); de *A. pacaas* pela presença de apenas um tubérculo subarticular no dedo IV da mão (dois tubérculos subarticulares no dedo IV da mão); de *A. gasconi* pela presença da linha ventrolateral clara completa (ausente ou irregular em *A. gasconi*).

Allobates **sp. nov.** pode ser confundido com *A. trilineatus* sensu stricto e *A. fuscillus* sensu stricto (Ferreira et al., em preparação) pela semelhança na coloração em espécimes vivos e preservados ou pela sobreposição do SVL em machos. Entretanto, *Allobates* **sp. nov.** difere de *A. trilineatus* sensu stricto pela marca paracloacal estreita (larga, redonda em forma de “vírgula” em *A. trilineatus* sensu stricto); pelo comprimento menor do dedo II da mão (2.8–3.3 mm *A. trilineatus* sensu stricto; 2.4–2.9 mm *Allobates* **sp. nov.**); pelo inchaço nos dedos II-III em machos (apenas o dedo III inchado em *A. trilineatus* sensu stricto e *A. fuscillus*) e pela

membrana basal entre os dedos II-IV dos pés (membrana basal somente entre os dedos III-IV em *A. trilineatus* sensu stricto e *A. fuscus*). Difere ainda de *Allobates fuscus*, pela média e amplitude do SVL. Machos e fêmeas de *Allobates* sp. nov. são menores [15.8 ± 0.4 (15.3–16.4mm); 16.0 ± 0.4 (15.6–16.5mm)], respectivamente, que machos e fêmeas de *Allobates fuscus* [16.9 ± 0.5 (15.9–17.6mm); 16.9 ± 1.0 (15.7–19.2)], respectivamente.

Canto de anúncio de *Allobates* sp. nov. é formado por duas notas (com intervalos regulares entre elas), emitidos solitariamente ou séries de dois a sete cantos, sendo, portanto, distinto de todas as espécies que emitem canto de anúncio composto por nota única, seja emitida continuamente: *A. caeruleodactylus*, *A. nidicola*, *A. subfolionidificans*; ou em séries/grupos ou trilhas de notas: *A. caldwella*, *A. fuscus*, *A. kamila*, *A. melanolaemus*, *A. nunciatus*; *A. paleovarzensis*, *A. sieggreenae*, *A. tinae*, *A. bacurau*, *A. grillisimilis*, *A. grillicantus*, *A. paleci* e *A. velocicantus*. Difere também daquelas espécies com cantos de anúncio mistos, com notas únicas emitidas continuamente e/ou pareadas, em trios ou grupo maior de notas: *A. tapajos* e *A. magnussoni*.

Canto de anúncio de *Allobates* sp. nov., tem frequência dominante de 4.996–5.965 Hz, o que o distingue de *A. bacurau* (5.890–6.340 Hz); *A. grillisimilis* (6.000–6.500 Hz); *A. melanolaemus* (3.840–4.560 Hz); *A. magnussoni* (4.273.0–4.704.6 Hz); *A. nidicola* (3.440–5.010 Hz); *A. nunciatus* (3.843–4.312 Hz) e *A. paleovarzensis* (4.050–4.930 Hz).

O canto de anúncio de *Allobates* sp. nov. pode ser confundido com o canto de anúncio de *A. flaviventris* e *A. trilineatus* sensu stricto pelo fato de ser composto por duas notas. Entretanto, *Allobates* sp. nov. difere destes por emitir cantos solitários e em séries de 2–7 cantos (somente em séries de 2–10 cantos em *A. flaviventris*; 6–11 em *A. trilineatus* sensu stricto. Veja Fig. 7A1-A2). Difere de *A. flaviventris* pela frequência dominante de 4.996–5.965 Hz (3.618–4.651 Hz em *A. flaviventris*) e distingue-se de *A. trilineatus* sensu stricto pelo intervalo de silêncio entre as séries de cantos (1.110–5.750 ms em *Allobates* sp. nov.; 7.040–46.650 ms em *A. trilineatus* sensu stricto. Veja Fig. 7A1-A2) e pela taxa de repetição (0.8 ± 0.2 em *Allobates* sp. nov.; 5.44 ± 0.20 ; 7.30 ± 0.61 ; 7.06 ± 0.14 nas gravações de três machos topotípicos de *Allobates trilineatus* sensu stricto – Jaramillo et al. 2021).

Girinos de *Allobates* sp. nov. são distintos dos congêneres endotróficos por não possuírem disco oral e espiráculo. Eles possuem papilas piramidais curtas no lábio anterior e papilas cilíndricas curtas no lábio posterior, distintos, portanto, daqueles que têm papilas longas no disco oral: *A. caeruleodactylus*, *A. grillisimilis*, *A. grillicantus*, *A. subfolionidificans* e *A.*

tapajos; e daqueles que possuem papilas piramidais no lábio posterior: *A. caldwella*, *A. carajas*, *A. kamilae*, *A. magnussoni* e *A. velocicantus*.

Girinos de *Allobates* **sp. nov.** têm um arranjo nas fileiras posteriores de dentes com $P1 \cong P2 > P3$. $P3$ corresponde a aproximadamente metade de $P1$ e $P2$, distinto dos girinos de *A. kamilae*, *A. nunciatus* ($P1$, $P2$ e $P3$ aproximadamente iguais), *A. palevarzensis* ($P2 > P1 > P3$) e *A. grillisimilis* ($P2=P1=P3$). Adicionalmente, a cauda dos girinos de *Allobates* **sp. nov.** tem pequenas manchas marrom claro distribuídas uniformemente, distinto de *A. caeruleodactylus* (manchas transversais grandes, marrom escuro); *A. caldwella*, *A. grillicantus* e *A. nunciatus* (manchas marrom escuro tamanhos variados). Girinos de *A. bacurau*, *A. conspicuus*, *A. flaviventris*, *A. fuscillus*, *A. gasconi*, *A. melanolaemus*, *A. ornatus*, *A. pacaas*, *A. paleci*, *A. sieggreenae*, *A. tinae*, *A. trilineatus* e *A. vanzolinius* não foram descritos ainda.

Descrição do holótipo. Medidas morfométricas são apresentadas na Tabela 2. Cabeça 6% mais larga que longa. Largura e comprimento da cabeça correspondem a 32% e 30% do SVL, respectivamente. Distância do olho à narina equivale a 68% da largura do olho. Largura do olho corresponde a 46% do comprimento da cabeça. Distância interorbital representa 92% da largura da cabeça. Tímpano corresponde a 38.6% do diâmetro do olho. Focinho semi truncado em vista dorsal. Narinas posicionadas lateralmente, na ponta do focinho, visíveis em vista lateral e ventral. *Canthus rostralis* redondo, ligeiramente côncavo, região loreal plana. Dentição presente, visível sob magnificação. Processo lingual médio ausente. Saco vocal simples, subgular, dobras laterais presentes na altura do ângulo da maxila.

Tubérculo palmar elíptico, conspícuo com diâmetro de 0,6mm. Tubérculo tenar, elíptico, conspícuo, com largura cerca de 80% do diâmetro do tubérculo palmar. Dedo III da mão com dois tubérculos subarticulares, redondos e protuberantes. O tubérculo basal ocupa 3/4 da largura da falange e o distal, 2/4. Um único tubérculo subarticular nos dedos I, II e IV da mão, tubérculo dedo I piramidal de largura similar à falange, dedo II redondo ocupa 3/4 da falange e dedo IV redondo ocupa 2/3. Ausência de tubérculos supranumerários. Ausência de franjas e membranas entre os dedos das mãos. Cume metacarpal presente. Excrescência do polegar ausente. Extremidade do dedo IV ultrapassa o tubérculo subarticular proximal, mas não alcança o tubérculo subarticular distal do dedo III, quando justapostos. Dedos II e III inchados. Forte inchaço preaxial do dedo III, evidente a partir da base à ponta do dedo (Fig. 6A). Dedo I maior que o dedo II. Comprimento relativo dos dedos das mãos: $IV < II < I < III$. Discos dos dedos das

mãos fracamente expandidos. Quilhas laterais presentes em todos os dedos da mão. No dedo I estende-se até a região anterior do tubérculo. Escudos dorsais digitais pareados presentes.

Comprimentos da coxa e da tíbia semelhantes, ambos 46% e 47% do SVL, respectivamente. Comprimento do pé 92% do comprimento da tíbia. Quilha tarsal tubercular, conspícua, curvada, estreitando-se em direção ao tubérculo metatarsal interno. Tubérculo metatarsal interno protuberante, elíptico. Tubérculo metatarsal externo pequeno e redondo, protuberante, menor que o diâmetro do interno. Cume metatarsal presente, alongado. Quilhas laterais presente em todos os dedos dos pés. Membrana basal entre os dedos II-IV dos pés; fraca entre os dedos II-III. Presença de tubérculos subarticulares arredondados, evidentes nos dedos dos pés, posicionados na altura das falanges, com a seguinte distribuição: dedos I e II, um; dedos III e V, dois; dedo IV, três. Discos dos dedos II-V dos pés bem expandidos e com pontas arredondadas. Disco do dedo I da mesma largura da falange, com ponta arredondada. Escudos dorsais digitais pareadas.

Em substância preservativa, superfície dorsal marrom, com grânulos e melanóforos estendendo-se da região interorbital à altura da virilha formando uma faixa retangular. Linha vertebral presente, estendendo-se da ponta do focinho à região próxima ao uróstilo (Fig. 5A). Focinho marrom claro. Linha dorsolateral clara presente, de coloração creme acinzentada, estendendo-se da região posterior ao olho à altura da virilha. Região dorsal dos braços, antebraços e mãos de coloração bege, com melanóforos; manchas irregulares de coloração marrom claro nas mãos (Fig. 5A). Superfície dorsal das coxas, tíbia, tarso e pés de coloração bege com alta concentração de melanóforos. Coxas sem barras transversais. Região anterior e posterior da coxa marrom claro em forma de faixa. Marcas paracloacais de coloração creme. Cada tíbia com uma barra transversal de coloração marrom (Fig. 5A). Tarso e pés com manchas marrom irregulares. Faixa lateral marrom escuro, circunda todo o corpo, estreita no focinho, alargando gradativamente, atingindo o máximo na região médio posterior do corpo (Fig. 5B). Linha ventrolateral clara presente, completa, do focinho à região inguinal (Fig. 5B). Região ventrolateral bege, com melanóforos. Barriga bege com grande quantidade de melanóforos uniformemente distribuídos (Fig. 5C). Peito com a cor de fundo bege, com grande quantidade de melanóforos. Garganta cinza claro, com grande quantidade de melanóforos, região periférica bege, com melanóforos espalhados uniformemente. Saco vocal com dobras à esquerda e à direita (Fig. 5C). Superfície ventral dos braços bege com poucos melanóforos. Superfície ventral dos antebraços de coloração marrom. Superfície de contato do antebraço de coloração creme sem melanóforos. Superfície ventral das coxas bege com pouco melanóforos. Superfície

anterior e posterior das coxas com manchas marrons. Superfície ventral da tíbia creme, melanóforos presentes em pouca quantidade e agrupados. Superfície de contato do tarso creme, sem melanóforos. Superfície ventral do tarso marrom (Fig. 5C). Palma das mãos e pés de coloração marrom claro (Figs. 5C; 6A, B).

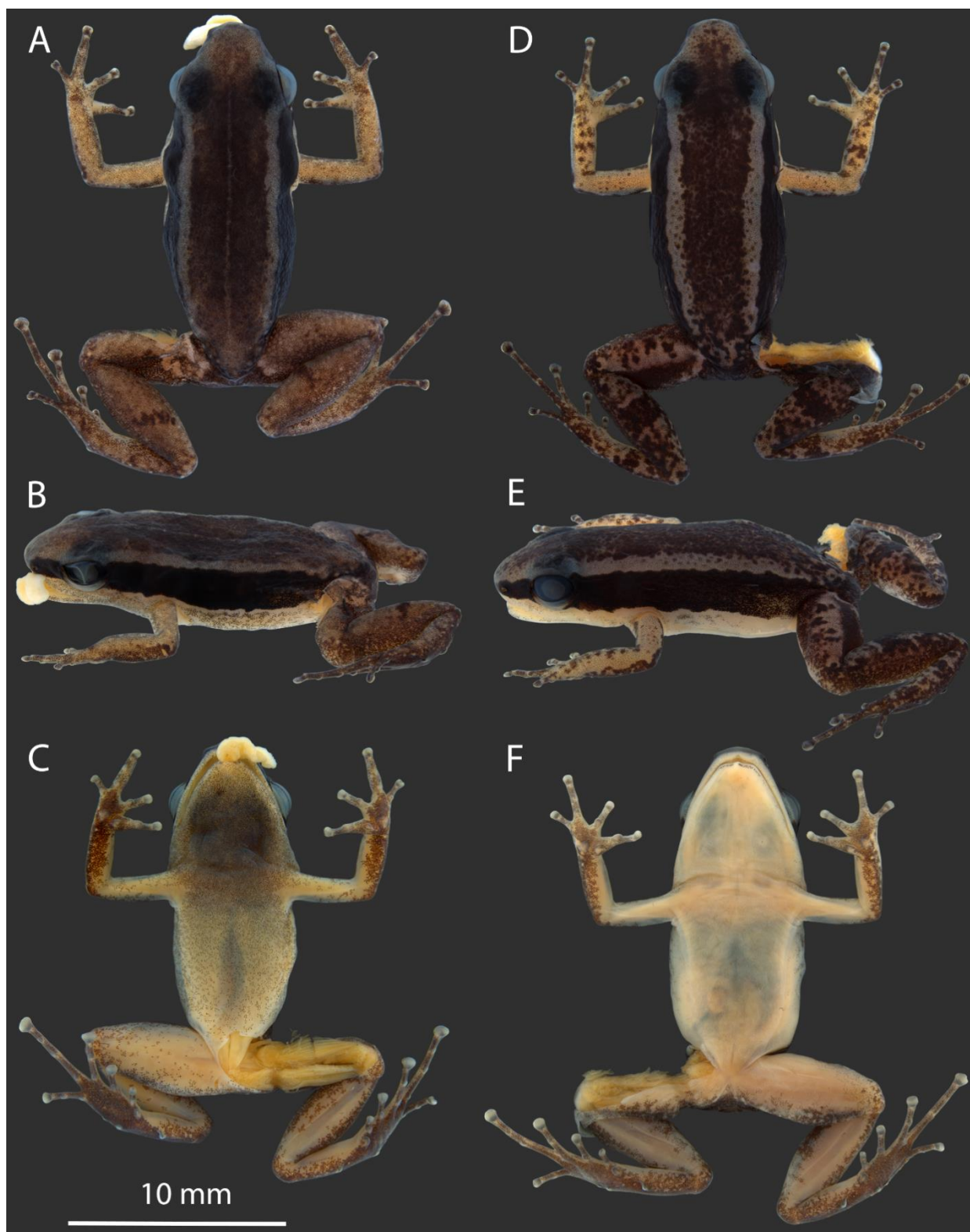


Figura 5. Vista dorsal, dorsolateral e ventral do macho holótipo INPA-H XXX (A–C) e fêmea parátipo INPA-H XXX (D–F) de *Allobates* sp. nov. Fotografias de JRDS.

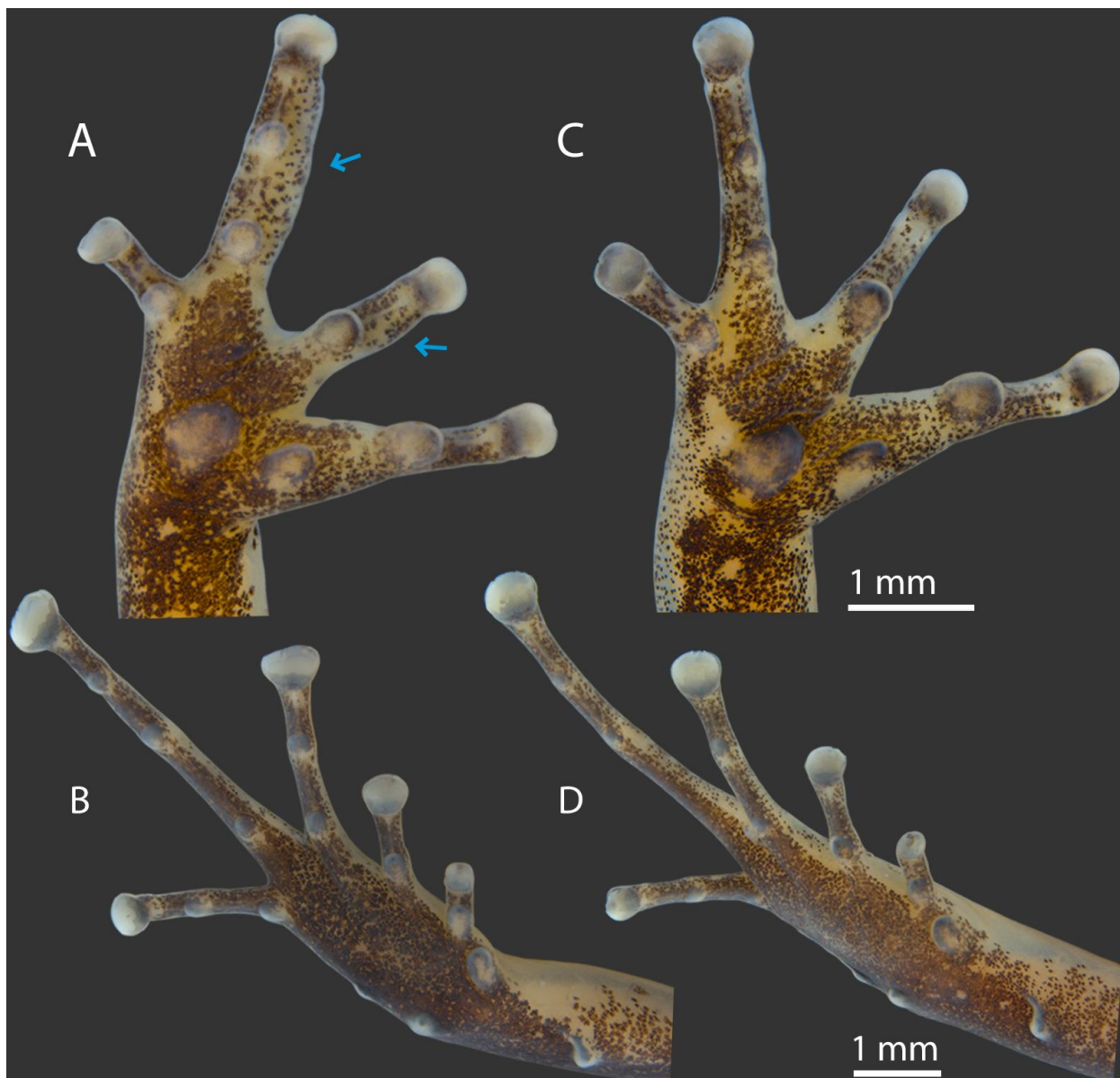


Figura 6. Vista ventral das mãos e pés direito de *Allobates* **sp. nov.** (A, B) macho holótipo INPA-H XXX (APL21378). (C, D) Fêmea parátipo INPA-H (APL21526). Note o inchaço preaxial nos dedos II-III do macho (setas azuis). Fotografias de JRDS.

Canto de anúncio

O canto de anúncio de *Allobates* **sp. nov.** é caracterizado pela emissão de duas notas, com intervalos curtos e regulares entre elas (Fig. 7B1). Os cantos podem ser solitários ou formar agrupamentos em series, com no máximo sete cantos (Fig. 7B1-B4). Tanto cantos quanto as séries de cantos possuem intervalos irregulares entre eles. A primeira nota de cada canto variou de 0,021 a 0,037s ($0,029 \pm 0,004$) e a segunda nota variou de 0,020 a 0,036s ($0,027 \pm 0,003$). O intervalo entre as notas variou de 0,013 a 0,035s ($0,0025 \pm 0,006$). O intervalo entre cantos variou de 0,21 a 0,66s ($0,35 \pm 0,10$) e o intervalo entre séries de cantos variou de 1,50 a 5,75s ($3,33 \pm 1,09$). A média da frequência dominante da primeira nota de cada canto é $5.467,6 \text{ Hz} \pm$

171,2 (4.995,7–5.943,2) e a média da frequência dominante da segunda nota é 5.645,9 Hz $\pm$ 174,7 (5.038,7–5.964,7) (Tabela 3).

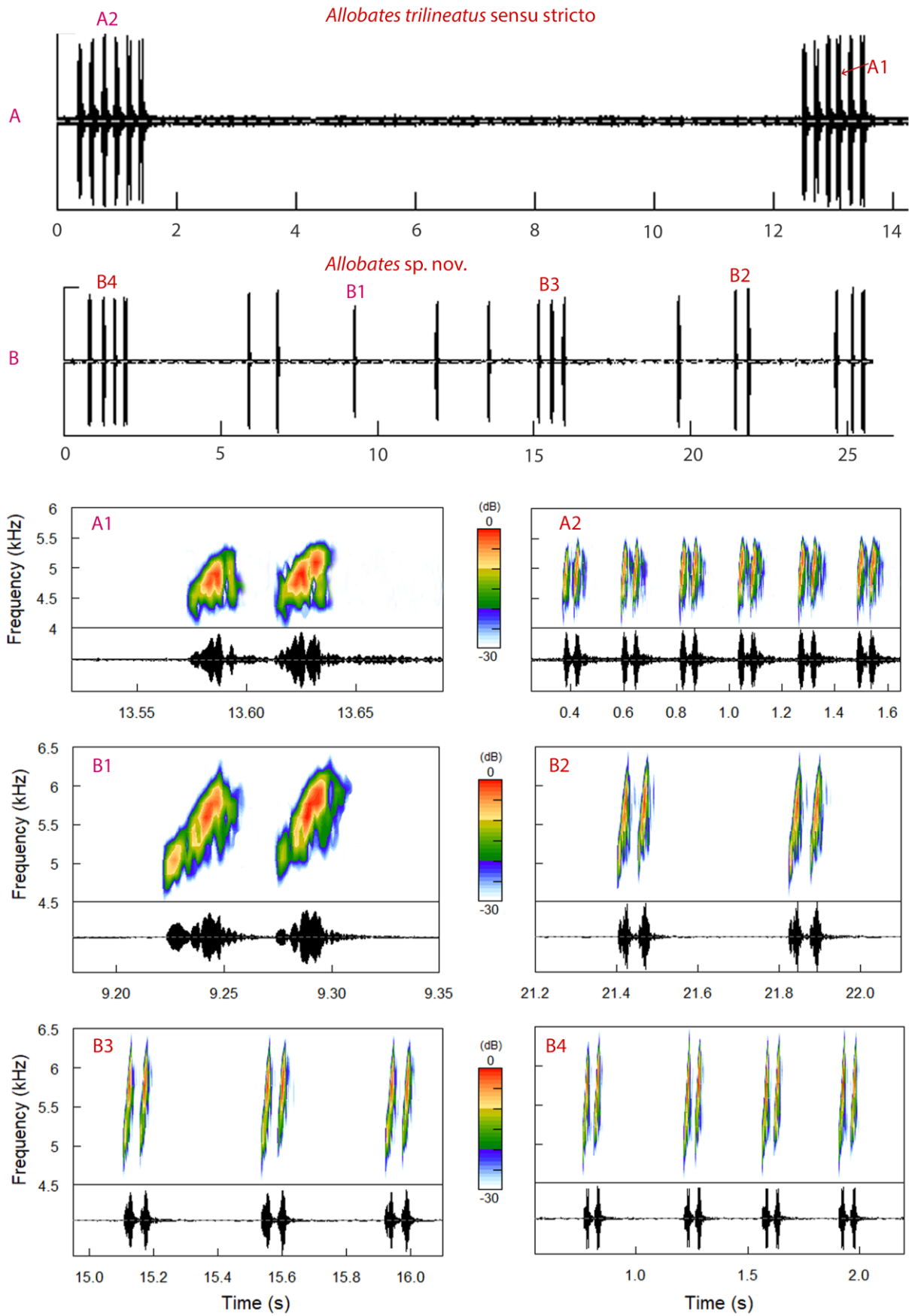


Figura 7. Cantos de anúncio de *Allobates trilineatus* sensu stricto (MCP14304) e *Allobates* **sp. nov.** (APL21379). (A) Oscilograma mostrando duas séries de seis cantos de *Allobates trilineatus* sensu stricto, separadas por um longo intervalo de silêncio, sem emissão de cantos solitários. Parâmetros espectrais e temporais são mais bem detalhados em A1, que consiste em um único canto pertencente a uma série e A2, série completa de seis cantos. (B) Oscilograma mostrando a emissão de cantos solitários (B1) e arranjado em séries de dois (B2), três (B3) e quatro (B4) cantos de *Allobates* **sp. nov.** Note que o canto de ambas as espécies é formado por duas notas (A1 e B1), com diferenças na amplitude da frequência. Outras diferenças incluem presença de cantos solitários e intervalo entre séries de cantos. Temperatura do ar: 26.7 °C para *Allobates trilineatus* sensu stricto e 25.5 °C para *Allobates* **sp. nov.** Abreviações: dB, decibéis; kHz, kilohertz; s, segundos.

Descrição dos girinos

Descrição dos caracteres quantitativos são baseados em girinos no estágio 34 (n=4; Tabela 4). Corpo ovóide em vista dorsal, redondo anterior e posteriormente, achatado em vista lateral. Comprimento do corpo (BL) 30–31% do comprimento total (TL) e comprimento da cauda 69–70% do TL; corpo mais largo que alto (BH 57–71% do BW) e mais comprido que largo (BW 71–78% do BL); HWLE 69–78–% do BW; focinho arredondado em vista lateral e dorsal; END 42–58% do ED; olhos dorsal e lateralmente dirigidos; IOD 52–66–% do HWLE. Narinas pequenas, dorsolateralmente localizadas e anterolateralmente dirigidas, visíveis em vista dorsal e lateral; distância entre narinas 42–52% do HWLE. Anel carnosos presente na margem interna das narinas, redondo, não ornamentado. Espiráculo único, sinistral, tubular, 0,42–0,46 mm de comprimento, atachado ventrolateralmente, Intestino enrolado, com seu eixo direcionado para o lado esquerdo do corpo, visível através da pele. Tubo anal destrá, 0,89–1,16 mm de comprimento. Altura máxima da cauda 2,44–2,77 mm. Nadadeira superior inicia cerca de 2 mm da inserção da cauda no corpo, borda rasa anteriormente, alcançando a altura máxima no terço médio posterior da cauda. Nadadeira superior mais alta que nadadeira inferior. Ponta da cauda suavemente acuminada. Musculatura da cauda em vista dorsal alcança 40–51% da largura do corpo; em vista lateral alcança 62–67% da altura do corpo. Disco oral localizado anteroventralmente, emarginado lateralmente em ambos os lados, transversalmente arredondado, 1,04–1,31 mm de largura, correspondendo a 29–38% da largura do corpo na altura do espiráculo. Lábio anterior com um grupo de seis papilas piramidais curtas, distribuídas em fileira única, sendo três em cada lado do lábio. Lábio posterior com uma única fileira de nove a 18 papilas cilíndricas curtas, de tamanho aproximadamente iguais (Fig. 8D, E). Papilas submarginais ausentes. Bainha da mandíbula superior em forma de arco, mais longa que a bainha inferior, sem corte mediano. Mandíbula inferior em forma de “V”, ligeiramente mais

larga que a mandíbula superior. Bordas da bainha superior e inferior serrilhadas; as serrilhas se estendem ao longo do comprimento inteiro de ambas as bainhas. Fórmula labial da fileira de dentes (LTRF) é 2(2)/3(1), com a fileira de dentes A-1 completa, medindo $0,70 \pm 0,13$ mm de comprimento; fileira de dentes A-2 medindo $0,72 \pm 0,01$ mm interrompida na região mediana por um intervalo de $0,45 \pm 0,05$ mm. Fileira P-1 medindo $0,76 \pm 0,08$ mm com um pequeno intervalo na região mediana. Fileiras P-2 e P-3 completas. P-2 medindo $0,70 \pm 0,05$ mm. P-3 medindo $0,36 \pm 0,01$ mm. $P-3 < P-2 < P-1$.

Superfície dorsal e lateral do corpo marrom claro (Fig. 8A, B). Região ventral translúcida, com melanóforos concentrados na mediana do corpo; órgãos internos visíveis através da pele (Fig. 8C). Musculatura da cauda de coloração creme, com manchas marrom claro de variados tamanhos e formas. Nadadeiras translúcidas com manchas marrom claro.

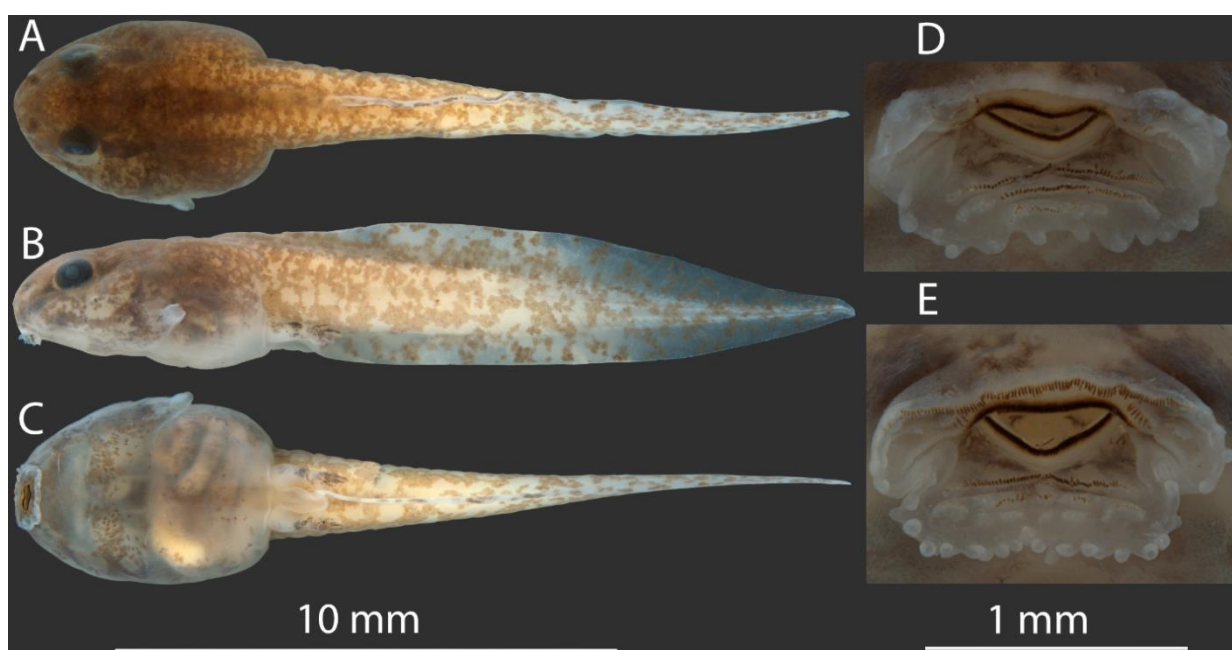


Figure 8 Girinos preservados de *Allobates* **sp. nov.**, no estágio 34 de Gosner (1960). Vista dorsal (A), lateral (B) e ventrolateral (C). Vistas frontais do disco oral (D, E). Fotografias de JRDS.

História natural

Allobates **sp. nov.** foi encontrado na serrapilheira de remanescentes de floresta primária. Até agora, não se conhece a real extensão de sua distribuição. Populações desta espécie foram

encontradas na região próxima à cidade de Manoel Urbano, Estado do Acre, nas áreas de reserva legal de uma propriedade particular, localizada na margem esquerda do rio Purus e na localidade Três Bocas, margem direita do rio Purus, abaixo da cidade de Manoel, à jusante. Estas populações estavam distribuídas próximas a pequenos cursos d'água, com presença de grande quantidade de poças temporárias, originadas durante o período chuvoso. Esta espécie utiliza folhas secas, enroladas ou dobradas, da serrapilheira, para deposição dos ovos. Machos vocalizam sobre folhas na serrapilheira ou empoleirados, em alturas de aproximadamente 10cm. Um evento de corte foi observado no período da manhã, entre 7:10hs e 8:52hs. A corte iniciou com aproximação da fêmea. O macho, ao percebê-la alterou a vocalização de anúncio para vocalização de corte, conduzindo-a até o sítio de oviposição (Fig. 9A). O macho entrou no sítio de oviposição e aguardou a fêmea, emitindo vocalização de corte e de anúncio alternadamente. Após a entrada da fêmea, ouviu-se grunhidos de baixa intensidade, e o macho subiu no dorso da fêmea, alcançando sua cabeça e realizando amplexo cefálico que durou menos de 1 min. Em seguida o macho abandonou o local. A fêmea permaneceu imóvel por alguns instantes e iniciou a oviposição. O macho voltou a vocalizar cinco minutos após sair do ninho. A fêmea permaneceu no sítio de oviposição por 92 minutos. A desova continha 14 ovos, de coloração cinza no polo animal e esbranquiçada no polo vegetativo, envolta em substância coloidal transparente, depositada em uma folha enrolada, na serrapilheira (Fig. 9B, C). A duração total do evento, a partir do momento em que a fêmea seguiu o macho até sair do ninho, foi de 102 minutos. A fecundação ocorre sem a presença do macho, uma vez que o macho abandona a desova antes da oviposição. Recolhida após a saída da fêmea, a desova se desenvolveu normalmente (Fig. 8D). Outra desova foi registrada neste mesmo dia, esta contendo com 13 ovos.

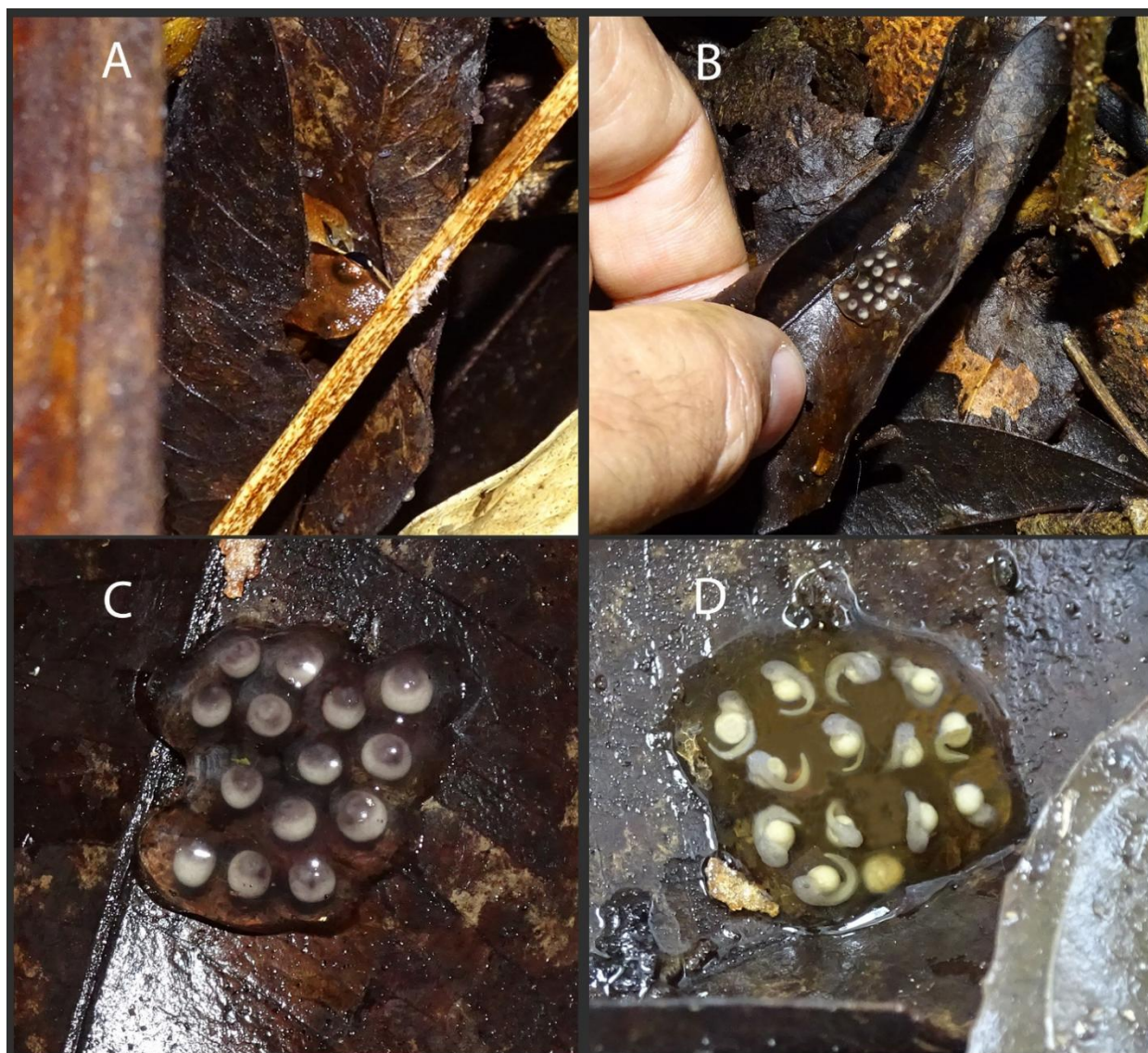


Figura 9. Aspectos da biologia reprodutiva de *Allobates* sp. nov. (A) Fêmea dentro da folha realizando a oviposição; (B e C) desova recém posta e (C) mesma desova após 7 dias de desenvolvimento, mostrando embriões no estágio 18-19, de Gosner (1960). Fotografias de JRDS.

Discussão

Descrições de espécies de anfíbios anuros realizadas até o início do século XXI, na maioria das vezes, focam apenas em aspecto morfológicos e trazem poucos detalhes sobre outros aspectos fenotípicos, sobre o posicionamento filogenético da espécie, bem como incertezas sobre seus âmbitos geográficos. Boa parte dessas descrições são simples e sucintas, seguindo o padrão da época, omitem informações importantes, como aspectos da história natural e características das larvas e até mesmo dados imprecisos ou equivocados de localização espacial (e.g., Boulenger, 1883; Melin, 1941; Morales, 2000), o que dificulta acesso posterior às áreas de ocorrência para coleta de material e confirmação da identidade das espécies. Esse

aspecto torna-se extremamente importante, atualmente, sobretudo em ecossistemas tropicais, dada a crescente perda e degradação de habitats florestais resultante da atividade humana e agravamento da crise climática (Davidson et al., 2012; Kruid et al., 2021; Young et al., 2016), os quais podem representar descaracterização ou perda da estrutura do habitat de localidades-tipo de espécies. Isto também ressalta a necessidade cada vez mais premente em refinar a resolução taxonômica das espécies, principalmente daquelas pertencentes a complexos de espécies crípticas, cujas distribuições amplas podem camuflar âmbitos geográficos restritos com elevado grau de endemismo e ameaça de extinção (Bickford et al., 2007).

O presente estudo reforça a ideia de que *Allobates trilineatus* representa um grande complexo de espécies crípticas do gênero. A recente descrição da espécie nominal (Jaramillo et al., 2021), bem como a presente investigação, servem de base para sanar problemas relacionados à delimitação de *Allobates trilineatus*, além de possibilitar a descrição de uma espécie-irmã, *Allobates sieeggrenae* (Gagliardi-Urrutia et al., 2021), bem como do táxon aqui descrito. Com base neste histórico e em outros estudos em andamento conduzidos por diferentes grupos de pesquisa, é bastante plausível que, por meio de abordagens integrativas, outras espécies sejam delimitadas e nomeadas neste e em outros complexos de espécies do gênero.

Sequências genéticas obtidas a partir de estudos como os de Grant & Rodriguez (2001), Melo-Sampaio et al., (2018), Grant et al., (2006; 2017), Réjaud et al., (2020), Jaramillo et al., (2021), Gagliardi-Urrutia et al., (2021), bem como as deste trabalho, foram essenciais para a compreensão de que o complexo *Allobates trilineatus* representa um clado muito mais rico do que se supunha. Isto pôde ser observado pelas espécies recuperadas como pertencentes ao clado *Allobates* aff. *trilineatus*, incluindo a descrita neste estudo, assim como, o clado *Allobates fuscillus* sensu stricto.

A distância genética observada entre *Allobates trilineatus* sensu stricto e *Allobates* **sp. nov.** (2,6%) pode ser considerada moderada em comparação a outras distâncias interespecíficas no gênero. Entretanto, além da diferença genética a nova espécie detém diferenças morfológicas diagnósticas importantes como inchaço no dedo II das mãos em machos, ausente em *Allobates trilineatus* sensu stricto e marca paracloacal estreita vs marca paracloacal larga em forma de “vírgula” em *Allobates trilineatus* sensu stricto. Além disso, a vocalização de anúncio da espécie aqui descrita é única por diferir especialmente quanto aos parâmetros estruturais dos cantos (emissão de cantos solitários e em séries de 2–7 cantos vs apenas séries de 7–11 cantos em *Allobates trilineatus* sensu stricto) e temporais referentes aos intervalos entre séries (1.110–5.750 ms em *Allobates* **sp. nov.** vs 7.040–46.650 ms em *Allobates trilineatus* sensu stricto). O

conjunto de caracteres diagnósticos obtidos neste estudo ressalta a importância de abordagens integrativas em taxonomia, em especial quando complexos de espécies fenotipicamente similares como este são investigados (Souza et al., in press).

De acordo com importante estudo revisivo de Jaramillo et al. (2021), *Allobates trilineatus* sensu stricto possui uma distribuição relativamente restrita em comparação ao que presumia-se, habitando áreas de terra firme na localidade Vila Munichs, no distrito de Yurimaguas, Peru. *Allobates* **sp. nov.** foi registrada até o momento em florestas ripárias em dois pontos ao longo do rio Purus, a cerca de 850 km de distância em linha reta da localidade-tipo de *Allobates trilineatus* sensu stricto. Além das várias prováveis barreiras geográficas naturais como os rios Purus, Taruacá, Liberdade, Juruá (no Brasil), bem como dos rios Ucayalli e Huallaga (no Peru), há fatores adicionais que podem influenciar a ocorrência e distribuição de espécies de *Allobates*, tais como estrutura da paisagem, vegetação e tipo de solo, tal como demonstrado para *Allobates femoralis* (Ferreira et al., 2018; 2020) ou como ocorre com a espécie irmã, *Allobates sieggreenae* (Gagliardi-Urrutia et al., 2021), cuja ocorrência está intimamente associada a florestas sob solos de areia branca. Embora nossa equipe de pesquisa ainda não tenha acessado regiões adjacentes, além dos pontos de ocorrência registrados, é provável que a nova espécie aqui descrita ocorra em habitats similares ao longo do rio Purus. Este cenário ressalta a importância de intensificação de esforços de amostragem em campo, particularmente em zonas de endemismo relativamente extensas, com baixa acessibilidade e pouco estudadas como é o caso da Inambari para que possamos fazer inferências mais assertivas sobre aspectos relevantes como padrões biogeográficos e de conservação acerca de *Allobates*.

Allobates **sp. nov.** é a segunda espécie descrita recentemente para o complexo *Allobates trilineatus*. Considerando que não foi possível acessar dados morfológicos, acústicos e comportamentais dos demais clados que compõem o complexo, os quais foram revelados através de análises moleculares, recomenda-se fortemente a continuidade das pesquisas na região buscando assim reunir dados provenientes de mais classes de caracteres e, consequentemente, evidências adicionais que auxiliem na hercúlea tarefa de revelar toda a diversidade deste grupo animal amplamente distribuído no sudoeste da Amazônia.

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Tabelas

Tabela 2. Medidas morfométricas (em milímetros) de indivíduos adultos de *Allobates* sp. nov.

Caracteres	Holótipo	Machos (n=11)	Fêmeas (n=8)
SVL	15.9	15.8 ± 0.4 (15.3–16.4)	16.0 ± 0.4 (15.6–16.5)
IO	4.7	4.7 ± 0.1 (4.5–4.9)	4.8 ± 0.2 (4.6–5.0)
HW	5.1	5.2 ± 0.2 (5.0–5.6)	5.1 ± 0.2 (4.9–5.4)
HL	4.8	4.9 ± 0.1 (4.7–5.2)	4.9 ± 0.2 (4.6–5.2)
IN	2.3	2.3 ± 0.1 (2.1–2.4)	2.4 ± 0.1 (2.2–2.6)
LL	7.3	7.1 ± 0.2 (6.8–7.6)	7.1 ± 0.3 (6.7–7.5)
TL	7.4	7.5 ± 0.2 (7.0–7.9)	7.3 ± 0.3 (6.9–7.7)
FL	6.8	6.9 ± 0.2 (6.6–7.3)	6.7 ± 0.3 (6.4–7.0)
UAL	4.0	4.2 ± 0.2 (3.9–4.4)	4.0 ± 0.2 (3.6–4.4)
FAL	3.5	3.6 ± 0.1 (3.4–3.8)	3.5 ± 0.1 (3.3–3.6)
HANDI	2.8	2.9 ± 0.1 (2.8–3.2)	2.9 ± 0.1 (2.7–3.1)
HANDII	2.7	2.7 ± 0.1 (2.4–2.9)	2.7 ± 0.1 (2.5–2.8)
HANDIII	3.8	3.8 ± 0.1 (3.6–4.0)	3.6 ± 0.2 (3.2–3.8)
HANDIV	2.1	2.3 ± 0.2 (2.0–2.5)	2.3 ± 0.2 (2.1–2.5)
WFD	0.5	0.5 ± 0.0 (0.5–0.6)	0.5 ± 0.0 (0.5–0.6)
WPF	0.5	0.5 ± 0.0 (0.4–0.5)	0.3 ± 0.0 (0.3–0.4)
DPT	0.6	0.6 ± 0.1 (0.5–0.7)	0.5 ± 0.1 (0.5–0.6)
WTT	0.5	0.4 ± 0.1 (0.3–0.6)	0.4 ± 0.1 (0.4–0.5)
WTD	0.8	0.7 ± 0.1 (0.7–0.9)	0.6 ± 0.1 (0.6–0.8)
TYM	0.9	0.9 ± 0.1 (0.8–1.0)	0.9 ± 0.1 (0.8–1.0)
EL	2.2	2.2 ± 0.1 (2.1–2.3)	2.2 ± 0.1 (2.0–2.3)
EN	1.5	1.5 ± 0.1 (1.3–1.7)	1.5 ± 0.1 (1.4–1.6)
SL	2.6	2.5 ± 0.1 (2.3–2.7)	2.5 ± 0.1 (2.4–2.6)
FL/TL	0.92	0.93 ± 0.03 (0.88–1.0)	0.92 ± 0.05 (0.88–1.00)
EN/EL	0.68	0.68 ± 0.03 (0.60–0.73)	0.67 ± 0.04 (0.61–0.75)
HL/SVL	0.30	0.31 ± 0.01 (0.29–0.32)	0.31 ± 0.01 (0.29–0.32)
HW/SVL	0.32	0.33 ± 0.01 (0.32–0.34)	0.32 ± 0.01 (0.31–0.34)

HL/HW	0.94	0.93 ± 0.02 (0.91–0.98)	0.97 ± 0.04 (0.91–1.02)
FAL/SVL	0.22	0.23 ± 0.01 (0.22–0.25)	0.22 ± 0.01 (0.20–0.23)
TL/SVL	0.47	0.47 ± 0.01 (0.45–0.49)	0.46 ± 0.02 (0.42–0.48)

Nota: Valores são apresentados como média $\pm$ desvio padrão, com variação entre parênteses.

Tabela 3. Caracterização temporal e espectral dos cantos de anúncio de *Allobates* sp. nov.

Voucher	SVL	C°	AC	CD	CI	IS	N1D	IPNI	N2D	N1LF	N1HF	N1DF	N2LF	N2HF	N2DF	RR
APL21342		21,0	94	0,080	0,33	3,8	0,027	0,030	0,026	4.920,7	5.740,1	5353,2	5.137,2	5.866,0	5.549,1	0,8
APL21376		23,7	31	0,080	0,30	2,8	0,027	0,029	0,024	5.091,4	6.129,9	5.633,1	5.398,3	6.239,1	5.861,3	1,0
APL21377		23,5	61	0,080	0,41	1,9	0,027	0,029	0,026	4.975,5	5.867,4	5.471,6	5.082,5	5.974,9	5.525,4	1,0
APL21378		23,7	31	0,080	0,53	3,3	0,030	0,024	0,026	5.091,2	5.873,3	5.439,3	5.337,6	6.081,9	5.602,9	0,6
APL21379		25,5	39	0,080	0,35	4,0	0,029	0,020	0,028	5.007,7	5.921,9	5.478,0	5.173,5	5.976,6	5.667,5	0,7
Unvouher		23,0	47	0,080	0,42	2,9	0,030	0,024	0,029	5.033,4	5.846,2	5.438,1	5.194,3	5.966,1	5.624,1	0,5
APL21529		22,0	59	0,080	0,31	3,8	0,030	0,020	0,028	4.937,0	5.946,3	5.463,0	5.141,2	5.995,7	5.693,4	0,7
Média		23,2	52	0,08	0,35	3,33	0,03	0,03	0,03	5.008,5	5.902,8	5.467,6	5.209,0	6.013,7	5.645,9	0,8
Sd		1,4	22	0,00	0,10	1,09	0,00	0,01	0,00	95,19	164,82	171,21	177,33	151,36	174,73	0,2
Max		25,5	94	0,09	0,66	5,75	0,04	0,04	0,04	5.155,2	6.276,7	5.943,2	5.942,2	6.313,5	5.964,7	1,0
Min		21,0	31	0,07	0,21	1,50	0,02	0,01	0,02	4.706,9	5.267,3	4.995,7	4.782,4	5.307,4	5.038,7	0,5

Note:

Valores para média, desvio padrão (Sd), máximo (Max) e mínimo (Min) são baseados em todos os cantos analisados. Abreviações adicionais: AC, número de cantos analisados; CD, duração do canto; CI, intervalo entre cantos; IS, intervalo entre séries de cantos; N1D, duração da 1ª nota; INPI, intervalo intra par de notas; N2D, duração da 2ª nota; N1LF, frequência mais baixa da 1ª nota; N1HF, frequência mais alta da 1ª nota; N1DF, frequência dominante da 1ª nota; N2LF, frequência mais baixa da 2ª nota; N2HF, frequência mais alta da 2ª nota; N2DF, frequência dominante da 2ª nota; RR, taxa de repetição.

Tabela 4. Medidas morfométricas (em milímetros) de sete girinos de *Allobates* **sp. nov.**, Estágios de Gosner 31, 33, 34 e 37, coletados no município de Manuel Urbano, estado do Acre, Brasil.

Medidas	Estágio 31 (n=1)	Estágio 33 (n=1)	Estágio 34 (n=4)	Estágio 37(n=1)
TL	13,02	14,11	15,86 ± 0,61 (14,95-16,21)	19,57
BL	4,03	4,20	4,81 ± 0,19 (4,54-4,96)	5,54
TAL	8,99	9,91	11,05 ± 0,43 (10,42-11,34)	14,03
BW	2,77	3,19	3,57 ± 0,11 (3,44-3,70)	4,03
BH	1,85	2,35	2,29 ± 0,20 (2,02-2,44)	2,77
HWLE	2,35	2,52	2,65 ± 0,15 (2,44-2,77)	3,11
TMW	1,34	1,43	1,62 ± 0,14 (1,43-1,76)	2,02
MTH	2,02	2,10	2,63 ± 0,14 (2,44-2,77)	3,02
TMH	1,18	1,18	1,47 ± 0,11 (1,34-1,60)	1,76
IOD	1,26	1,43	1,53 ± 0,16 (1,43-1,76)	1,93
IND	1,09	1,18	1,22 ± 0,05 (1,18-1,26)	1,34
END	0,38	0,29	0,42 ± 0,06 (0,34-0,46)	0,50
NSD	0,29	0,29	0,37 ± 0,12 (0,25-0,50)	0,29
ED	0,71	0,76	0,82 ± 0,02 (0,80-0,84)	0,88
SS	2,94	2,86	3,21 ± 0,17 (3,02-3,44)	3,44
VTL	0,69	0,79	1,04 ± 0,13 (0,89-1,16)	1,51
STL	0,34	0,42	0,45 ± 0,02 (0,42-0,46)	0,46
ODW	1,02	1,09	1,21 ± 0,13 (1,04-1,31)	1,43
PL	0,17	0,25	0,27 ± 0,03 (0,24-0,30)	0,34
AL	0,27	0,37	0,38 ± 0,02 (0,37-0,40)	0,42
GAP	0,44	0,45	0,45 ± 0,05 (0,42-0,49)	0,60
A1	0,62	0,87	0,70 ± 0,13 (0,60-0,79)	1,21
A2	0,62	0,69	0,72 ± 0,01 (0,71-0,72)	0,79
P1	0,62	0,45	0,76 ± 0,08 (0,67-0,82)	0,76

P2	0,59	NA	0,70 ± 0,05 (0,67-0,76)	0,76
P3	0,27	NA	0,36 ± 0,01 (0,35-0,37)	NA
UJW	0,17	0,17	0,21 ± 0,08 (0,17-0,34)	0,34
UJL	0,60	0,47	0,60 ± 0,08 (0,54-0,71)	0,72

Note: Para estágios com mais de um girino, valores representam a média ± desvio padrão (mínimo–máximo). Abreviações: n, tamanho da amostra; NA, não medido. Veja o texto principal para abreviações das medidas morfométricas.

Appendix 1. Espécimes, vouchers e respectivos números de acesso das sequências utilizadas nas análises filogenéticas.

Espécies	Voucher	12S	16S	ND1	COI	Cytb
<i>Allobates</i> aff. <i>granti</i>	125PG	JN690205	JN690931			
<i>Allobates</i> aff. <i>magnussoni</i>	977126	MT627173	MT627173	MT627173	MT627173	MT627173
<i>Allobates</i> aff. <i>melanolaemus</i>	MTR28013	MT627203	MT627203	MT627203	MT627203	MT627203
<i>Allobates</i> aff. <i>olfersioides</i> 1	MTR17821		KDQF01003353			
<i>Allobates</i> aff. <i>olfersioides</i> 2	MTR16435	MT627202	MT627202	MT627202	MT627202	MT627202
<i>Allobates</i> aff. <i>olfersioides</i> 3	JFT959		KDQF01002701			
<i>Allobates</i> aff. <i>tapajos</i> 1	MTR10084	MT627197	MT627197	MT627197	MT627197	MT627197
<i>Allobates</i> aff. <i>tapajos</i> 2	AF1906	MT627175	MT627175	MT627175	MT627175	MT627175
<i>Allobates</i> aff. <i>tinae</i> 1	MPEG13397	DQ502213	DQ502213		DQ502900	DQ502648
<i>Allobates</i> aff. <i>trilineatus</i> Clado 1	FGZC3247	MT627185	MT627185	MT627185	MT627185	MT627185
<i>Allobates</i> aff. <i>trilineatus</i> Clado 1	MJH7477	DQ502118	DQ502118		DQ502826	DQ502550
<i>Allobates</i> aff. <i>trilineatus</i> Clado 1	FGZC3272		MT524142			
<i>Allobates</i> aff. <i>trilineatus</i> Clado 2	MHNC8367		MT524173			

<i>Allobates</i> aff. <i>trilineatus</i> Clado 2	MHNC8371		MT524174		
<i>Allobates</i> aff. <i>trilineatus</i> Clado 3	KU215172	DQ501986	DQ501986	DQ502712	DQ502411
<i>Allobates</i> aff. <i>trilineatus</i> Clado 3	KU215175	DQ501998	DQ501998	DQ502720	DQ502423
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MPEG12356	DQ502050	DQ502050	DQ502775	DQ502481
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MPEG12359	DQ502051	DQ502051	DQ502776	DQ502482
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MPEG12360	DQ502190	DQ502190	DQ502889	DQ502626
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	OMNH36027	DQ502194	DQ502194	DQ502892	DQ502630
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	OMNH36026	DQ502198	DQ502198	DQ502894	DQ502634
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MCP13618		KY886592	KY886606	KY886635
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MCP13639		KY886591	KY886605	KY886634
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MNRJ91676		KY886595		
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MTR28420		KY886593	KY886607	KY886636
<i>Allobates</i> aff. <i>trilineatus</i> Clado 4	MTR28494		KY886594	KY886608	KY886637

<i>Allobates</i> aff. <i>trilineatus</i>	LSUMZ13690	EU342562	EU342562			
Clado 4						
<i>Allobates</i> aff. <i>undulatus</i>	AMNHA159139	DQ283044	DQ283044		DQ502756	DQ502459
<i>Allobates algorei</i>	TNHCFS5551	HQ290950	HQ290950	HQ290950		HQ290530
<i>Allobates amissibilis</i>	PK3798	MT627204	MT627204	MT627204	MT627204	MT627204
<i>Allobates bacurau</i>	INPAH35401		KU195701			
<i>Allobates caeruleodactylus</i>	MTR10227	MT627199	MT627199	MT627199	MT627199	MT627199
<i>Allobates caldwella</i>	MPEG13826	DQ502099	DQ502099			DQ502531
<i>Allobates carajas</i>	BM163	MT627183	MT627183	MT627183	MT627183	MT627183
<i>Allobates chalcopis</i>	Alca1	MT627182	MT627182	MT627182	MT627182	
<i>Allobates</i>						
<i>conspicuus/subfolionidifica</i>	FGZC3279	MT627186	MT627186	MT627186	MT627186	MT627186
<i>ns</i>						
<i>Allobates crombiei</i>	AF1097	MT627174	MT627174	MT627174	MT627174	MT627174
<i>Allobates femoralis</i>	AF3224	MT627179	MT627179	MT627179	MT627179	MT627179
<i>Allobates femoralis</i> SS	AfemShucv3a	DQ523001	DQ523072			DQ523142
<i>Allobates flaviventris</i>	HJ545	MT627192	MT627192	MT627192	MT627192	MT627192
<i>Allobates fratisenescus</i>	QCAZ54377		MF624172			MF614174
<i>Allobates fuscillus</i> SS	APL23380		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23381		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23387		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23393		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23334		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23335		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23503		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23505		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23507		XXXXXX			
<i>Allobates fuscillus</i> SS	APL23509		XXXXXX			

<i>Allobates fuscus</i> SS	MCP13628		KY886585		KY886602	KY886626
<i>Allobates fuscus</i> SS	MNRJ91663		KY886583		KY886600	KY886624
<i>Allobates fuscus</i> SS	MNRJ91664		KY886584		KY886601	KY886625
<i>Allobates fuscus</i> SS	MNRJ91666		KY886586		KY886603	KY886627
<i>Allobates fuscus</i> SS	MNRJ91668		KY886587		KY886604	KY886628
<i>Allobates fuscus</i> SS	MCP10212		MF624184			
<i>Allobates gascon</i> SL1	APL14410		KJ747333			
<i>Allobates gascon</i> SL2	APL23940		OQ297604			
<i>Allobates gascon</i> SL3	HJ480	MT627191	MT627191	MT627191	MT627191	MT627191
<i>Allobates gascon</i> SL4	MNRJ91683		KY886574			KY886615
<i>Allobates gascon</i> SL5	MPEG13003	DQ502052	DQ502052		DQ502777	DQ502483
<i>Allobates gascon</i> SS	APL23345		ON997555			
<i>Allobates gascon</i> SS	GGU684		MT524137			
<i>Allobates goianus</i>	SAMA8574	MT627207	MT627207	MT627207	MT627207	MT627207
<i>Allobates granti</i>	AF1998	MT627176	MT627176	MT627176	MT627176	MT627176
<i>Allobates grillicantus</i>	MPEG43046		MW220039			
<i>Allobates grillisimilis</i>	MTR12749	MT627200	MT627200	MT627200	MT627200	MT627200
<i>Allobates hodli</i>	ABU2194		KX044279			
<i>Allobates humilis/pittieri</i>	CVULA5690	KJ940454	KJ940454			
<i>Allobates insperatus/juami</i>	JMP1703	MT627193	MT627193	MT627193	MT627193	MT627193
<i>Allobates juanii/ranoides</i>	ARA2394	DQ502271	DQ502271		DQ502933	DQ502702
<i>Allobates kamillae</i>	HJ285	MT627189	MT627189	MT627189	MT627189	MT627189
<i>Allobates kingsburyi</i>	QCAZ16523	AY364549	HQ290963			HQ290541
<i>Allobates magnussoni</i>	BM168	MT627184	MT627184	MT627184	MT627184	MT627184
<i>Allobates marchesianus</i>	AJC2498	MT627180	MT627180	MT627180	MT627180	MT627180
<i>Allobates masniger</i>	MTR10155	MT627198	MT627198	MT627198	MT627198	MT627198
<i>Allobates melanolaemus</i>	NMP6V711404		MT524148			
<i>Allobates nidicola</i>	MPEG13821	DQ502101	DQ502101			DQ502533

<i>Allobates niputidea</i>	MUJ3520	DQ502272	DQ502272		DQ502934	DQ502703
<i>Allobates nunciatus</i>	MPEG36777	MT627196	MT627196	MT627196	MT627196	MT627196
<i>Allobates olfersioides</i>	MNRJ79897	MF624178	MF624178			MF614175
<i>Allobates ornatus</i>	MHNSM22863		EU342550			
<i>Allobates pacaas</i>	MZUSP158938		MT076999			
<i>Allobates paleovarzensis</i>	JMP2196	MT627194	MT627194	MT627194	MT627194	
<i>Allobates sieggreenae</i> SS	JMP2313	MT627195	MT627195	MT627195	MT627195	MT627195
<i>Allobates sieggreenae</i> SS	MCP14533		MW293942			
<i>Allobates sieggreenae</i> SS	MCP 14539		MW293941			
<i>Allobates sieggreenae</i> SS	CRBIIAP1740		MW293943			
<i>Allobates</i> sp. Huanuco	FGZC3348	MT627187	MT627187	MT627187	MT627187	MT627187
<i>Allobates</i> sp. Neblina	MTR15537	MT627201	MT627201	MT627201	MT627201	MT627201
<i>Allobates</i> sp. nov.	APL21482		XXXXXX			
<i>Allobates</i> sp. nov.	APL21484		XXXXXX			
<i>Allobates</i> sp. nov.	APL21510		XXXXXX			
<i>Allobates</i> sp. nov.	APL21516		XXXXXX			
<i>Allobates</i> sp. nov.	APL21529		XXXXXX			
<i>Allobates</i> sp. nov.	APL21490		XXXXXX			
<i>Allobates</i> sp. nov.	APL21491		XXXXXX			
<i>Allobates</i> sp. nov.	APL21492		XXXXXX			
<i>Allobates sumtuosus</i>	AF2212	MT627177	MT627177	MT627177	MT627177	MT627177
<i>Allobates talamancae</i>	QCAZ35236	MT627205	MT627205	MT627205	MT627205	MT627205
<i>Allobates tapajos</i>	MJH3973	DQ502110	DQ502110		DQ502820	DQ502542
<i>Allobates tinae</i>	HJ298	MT627190	MT627190	MT627190	MT627190	MT627190
<i>Allobates trilineatus</i> SS	AF4493		MT524111			
<i>Allobates trilineatus</i> SS	MCP14304		MW293944			
<i>Allobates trilineatus</i> SS	MCP14306		MW293945			

<i>Allobates trilineatus</i> SS	MCP14309		MW293946			
<i>Allobates trilineatus</i> SS	MCP14317		MW293947			
<i>Allobates undulatus</i>	AJC3040	MT627181	MT627181	MT627181	MT627181	MT627181
<i>Allobates velocicantus</i>	MCP10187/88	MF624181	MF624181			MF614178
<i>Allobates zaparo</i>	USNM546405	DQ502026	DQ502026		DQ502752	DQ502455
<i>Ameerega hahneli</i>	AF2673	MT627178	MT627178	MT627178	MT627178	MT627178
<i>Anomaloglossus stepheni</i>	MJH3928	DQ502107	DQ502107		DQ502818	DQ502539
<i>Aromobates</i>	TNHCFS5541/AMNH					
<i>saluensis/nocturnus</i>	A130042	HQ290970	HQ290970	HQ290970	DQ502860	DQ502592
<i>Colostethus brachistriatus</i>	CZPDUV4603	MF624204	MF624204		MF614304	MF614198
<i>Dendrobates auratus</i>	MVZHerp149723	JX564862	JX564862	JX564862	JX564862	JX564862
<i>Epipedobates boulengeri</i>	UMMZ227952/QCAZ 16574	HQ290997	HQ290997	HQ290997	DQ502742	DQ502447
<i>Leucostethus fugax</i>	QCAZ16513	HQ290958	HQ290958	HQ290958		HQ290538
<i>Mannophryne collaris</i>	FS5523	MT627188	MT627188	MT627188	MT627188	MT627188
<i>Phyllobates terribilis</i>	TNHC64420/AMNHA 118566	HQ291006	HQ291006	HQ291006	DQ502861	DQ502593
<i>Rheobates palmatus</i>	RHEOPALM	MT627206	MT627206	MT627206	MT627206	MT627206
<i>Silverstoneia</i>	TNHCFS4942/MAR33					
<i>nubicola/erasmios</i>	6	HQ290966	HQ290966	HQ290966	MF614333	MF614237

CONSIDERAÇÕES FINAIS

Estamos diante de um grande desafio que é conhecer a biodiversidade do planeta antes que ela desapareça frente aos elevados índices de modificação de habitats e consequente extinção de espécies. Desmatamento, incêndios florestais, poluição das águas e uso de combustíveis fósseis, são algumas das atividades humanas que promovem as mudanças climáticas e da paisagem e que ameaçam a persistência das espécies e de suas linhagens componentes. Diante deste cenário, não existe um horizonte temporal que nos permita desperdiçar tempo, frente ao gigantesco problema que é descrever cerca de nove milhões de espécies (Mora et al. 2011) antes que sejam extintas. Neste contexto, a taxonomia integrativa tem um papel fundamental em promover novas abordagens, com diferentes interfaces para delimitação e descrição de novas espécies. Desde os tempos de Linnaeus que revolucionou a taxonomia, não via-se com tanta esperança o alcance deste objetivo.

De modo geral, os resultados obtidos nesta tese ampliam o conhecimento sobre a diversidade de sapos do gênero *Allobates*, sobretudo espécies crípticas que se distribuem na região mais rica e mais ameaçada do planeta. Ao integrar dados moleculares, bioacústicos e morfológicos de adultos e larvas, novas linhagens foram reveladas ampliando-se o conhecimento taxonômico sobre este grupo altamente diverso. No primeiro capítulo, descrevemos uma nova espécie de *Allobates* para a região do Juruá, considerada extremamente importante para conservação de diversos grupos biológicos. *Allobates velocicantus* é pronta e facilmente diferenciada das demais espécies de *Allobates* genética e morfológicamente, mas principalmente pelo canto de anúncio composto de 66-138 notas. Além disso, representa um modelo excelente para estudos ecológicos e adaptações fisiológicas à radiação solar em ovos de *Allobates*. No segundo capítulo, descrevemos uma nova espécie de *Allobates* para a região de cabeceiras do rio Purus, erroneamente classificada e confundida com *Allobates gasconi* em diversos locais na Amazônia. Além das diferenças morfológicas e de coloração, ao adicionarmos novas sequências genéticas e utilizarmos delimitadores moleculares, clareamos a filogenia do clado *caeruleodactylus* e demonstramos que *Allobates gasconi* sensu stricto e a nova espécie são bastante distintas. No terceiro capítulo, descrevemos uma nova espécie pertencente ao complexo *Allobates trilineatus*, amplamente distribuído na Amazônia e que tem sua diversidade subestimada, com várias linhagens aguardando descrição formal. Por fim, as publicações resultantes da tese, bem como todos os demais dados brutos depositados em repositórios públicos, certamente auxiliarão em futuros processos de delimitação de linhagens,

resolução taxonômica e classificação sistemática de *Allobates* e de toda a megadiversidade do sudoeste da Amazônia.

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